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# Detection of cocaine and its major metabolites in bone following outdoor decomposition after chronic cocaine administration using 2D-LC/MS/MS

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# DETECTION OF COCAINE AND ITS MAJOR METABOLITES IN BONE FOLLOWING OUTDOOR DECOMPOSITION AFTER CHRONIC COCAINE ADMINISTRATION USING 2D-LC/MS/MS

by

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**DETECTION OF COCAINE AND ITS MAJOR METABOLITES IN BONE FOLLOWING OUTDOOR DECOMPOSITION AFTER CHRONIC COCAINE ADMINISTRATION USING 2D-LC/MS/MS**

**MALORIE ANN MELLA**

ABSTRACT

In the field of forensic toxicology, several challenges exist with quantification analysis of cocaine and metabolites in post mortem samples. Cocaine can prove difficult to detect and quantify in blood, urine, and soft tissues following extensive decomposition. Alternative matrices, such as hair, nails, and bone could prove useful in detecting chronic drug use in post-mortem toxicology cases. Detection and quantification of drugs in complex matrices is difficult to accomplish due to time-consuming extraction processes, and inability to detect an analyte at trace levels. Further, analysis of drugs in hard tissues, such as hair and bone, has only been attempted in recent years. Even fewer studies have investigated detection of drugs following decomposition of remains, specifically outdoor decomposition. The objective of this study was to develop a robust extraction and clean up methodology, in which a homogenization step precedes, to efficiently extract drugs from complex matrices, reach a target limit of detection (LOD) and to maintain instrument performance using multidimensional chromatography. Multi-dimension chromatography platform such as two dimensional liquid chromatography tandem mass spectrometry ( 2D-LC/MS/MS,) offers options not compatible with single dimension

units. With large volume injection capabilities of aqueous and organic extracts, the analytical process be reduced from multiple hours to minutes.

All rat specimens used for this study fell under an Institutional Animal Care and Use Committee (IACUC) protocol. The rodents underwent a 10-12 weeks chronic intravenous self-administration of cocaine. This was followed by a six-week period of abstinence, followed again by a three-week period of cocaine self-administration before being euthanized. Average daily dosages for each rat fell within a range of 13-19 mg/kg. A total of 14 cocaine positive rats were placed outside and above ground in the Boston University Forensic Anthropology Outdoor Research Facility (Holliston, MA, U.S.A) for a period of 12 months. All recoverable skeletal samples were collected for testing. Drug free control rat bones were also acquired by placing drug-free rats outdoors, above ground, until full decomposition occurred. In this study, a method analyzing cocaine and its major metabolites benzoylecgonine and ecgonine methyl ester was developed.

After homogenization of whole bones, the extraction process was performed using a mixed mode reversed-phase/ion exchange sorbent. The use of a 2D LC/MS/MS technology eliminates the need for a lengthy evaporation step in the extraction method. The chosen 2D LC/MS/MS used in this application was identified using a 6x6 automated method development protocol. The manual extraction of the bone samples was completed in less than an hour. The analysis was performed using 100µL of the final organic solvent (MeOH) extracts.

The limit of quantitation (LOQ) for cocaine and benzoylecgonine was measured at 0.05ng/g (0.05ng/mL or 50pg/g) of sample material and the LOQ for ecgonine methyl ester was measured at 0.1ng/g (0.1ng/mL or 100pg/g). The extraction method for cocaine proved to give a linear dynamic range of 2.5 orders of magnitude (0.05ng/g to 10ng/g with an $R^2 = 0.998$.

The micro extraction protocol combined with a multi-dimension chromatography used in this study decreased sample preparation time without sacrificing the quality seen with current single dimension chromatography techniques. The procedure developed in this study can be utilized on bone and completed in less than an hour before injection into the 2D-LC/MS/MS system.

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| | |
|---|---|
| 2D | Two-dimensional |
| fg | femtogram |
| g | gram |
| LC | Liquid Chromatography |
| LOD | Limit of detection |
| LOQ | Limit of quantitation |
| mL | Milliliter |
| MS | Mass Spectrometry |
| ng | nanogram |
| pg | Picogram |

# 1. INTRODUCTION

## 1.1 Post-mortem Toxicology

In forensic cases, one of the most vital tools a medical examiner has is toxicological analysis. By testing blood, urine, gastric fluid, vitreous fluids, and less often brain and other tissues, one may be able to determine cause and/or manner of death or if the victim was poisoned or impaired at the time of death. The decomposition process of the human body poses a major hindrance to toxicological analysis. The process of decomposition includes the onset of autolysis, putrefaction and postmortem redistribution. Autolysis occurs due to naturally present enzymes in the body, in particular the pancreas and gastric mucosa; and putrefaction is destruction of cells and tissues by microorganisms. Whether due to the natural metabolic actions of the human body, stability of the drug itself, alkaline conditions of a decomposing body, chemical changes, bacteria or enzymatic activity, severe putrefaction and autolysis of a human body will make any analysis of drugs or poisons in tissues and body fluids very difficult (1). Many studies have shown that the ability to detect and accurately quantify target analytes such as drugs of abuse or pesticides in body tissues and fluids severely decline the longer the decomposition process (1).

In some cases, the victim's body may be so badly decomposed, to the point of complete skeletonization, prohibiting toxicological screening. In these cases, investigators must turn to alternative tissues for testing such as hair, nails, and bone. However, these tissues are less often employed due to the difficulties associated with sample preparation.

## 1.2 Alternative matrices for toxicological testing

### 1.2.1 Hair as an alternative matrix

Nails and hair are examples of keratinized tissues which are known to retain drugs and heavy metals for longer periods of time. These harder tissues are also more resistant to decomposition and are more durable samples. Hair specimens, in particular, are very useful to test for chronic drug use or exposure to heavy metals or toxins (1). Capillary blood surrounding the hair germination, skin/gland secretions, and external environment factors all provide entry points for substances to reach the growing hair matrix (2). The greatest advantage of drug testing keratinized tissues such as hair or nails over typical bodily fluids is stability of drugs in hair and nails. Samples can typically be stored at room temperatures for long periods of time without major degradation of drugs which have been incorporated into the matrix (3). Additionally, segmental hair analysis can provide a retroactive drug history and provide long-term history of drug use when there is no prior history or confirm self-reported histories. For example, forensic analysis of 88-cm-long cut of dreadlocks of a deceased individual demonstrated increased usage of heroin over time followed by a period of abstinence before further toxicology testing of fluids indicated heroin use—which assisted officials in concluding that cause of death was an accidental heroin overdose (2). This makes hard matrices very useful in both fresh postmortem cases and after soft tissues has decomposed beyond drug testing capabilities. The disadvantage of drug testing in hair is that results can only provide a historical account of drug usage (4). Unlike blood or plasma concentrations, the concentration of drug in the hair itself is not an accurate indicator of the dosage of drug consumed.

### 1.2.2 Bone as an alternative matrix

Bone is a hard tissue composed of hydroxyapatite and collagen, which further develops into compact and cancellous bone. Areas of cancellous bone constitute sites of red marrow which produce red and white blood cells and platelets. It also contains yellow marrow which is a reserve of fat cells found in the medullary cavity, the hollow inside of bone shafts. The periosteum, a thin tissue which covers the outsides of bones, is a tough, vascularized membrane that nourishes bone. The more porous cancellous bone receives nutrition from surrounding blood vessels in the body. Compact bone consists of cells with Haversian system, which consist of canals through which blood, lymph, and nerve fibers pass. Bone is also in a constant state of remodeling, which incorporates bone building cells. Clearly, bone and bone marrow is highly vascularized and nourished in the body, yet they are well protected from the environment. It survives intact for a greater length of time than any soft tissue in the body. Bone marrow, also used for toxicological testing, is protected by both the periosteum around the bone and the bone itself. It is safe from putrefaction as the body decomposes, however the hard bone itself is what persists for thousands of years intact. Therefore, it is a suitable option for toxicological screening. It is an obvious choice for forensic testing when human remains are in an extreme state of decomposition.

An additional disadvantage to using hair for toxicological analysis is that it can be extremely variable from person to person. Many factors can affect drug incorporation into hair and subsequent detection such as chemical treatments, melanin content, and pigment content in hair (5), all factors which vary from person to person. Bone is not

exposed to the environment as easily as hair. It is not dyed or altered. During decomposition, most environmental and putrefaction byproducts will not penetrate every layer of bone. Hair is decidedly more fragile when exposed to such extreme an environment.

Until recent years, it was unclear as to whether drugs are distributed in skeletal tissues and whether they can be detected at various time points after death. Bone does not grow the way hair does, and the way in which drugs are incorporated into the bone itself is not specifically known. There are many routes in which drugs may incorporate itself into the bone material itself. Bone formation takes place throughout one's entire life—bone formation or resorption cells are constantly working to build, remove and replace bone cells according to an individual's lifestyle. The first way is that drugs travel through the body, and by way of the highly vascularized periosteum, surrounding blood vessels, or Haversian canals, they are absorbed into the available surfaces or incorporated into the bone building cells responsible for remodeling. It is also possible that drugs are absorbed into bone surfaces during decomposition when there is total breakdown of soft tissues. Regardless of how drugs enter the bones themselves, it has become clear that it is possible to detect drugs of abuse in bone samples (6-17).

Studies utilizing rat bones or porcine bones have shown drugs of abuse such as ketamine, diazepam, citalopram, amitriptyline, colchicines, meperidine, tramadol, and pentobarbital are still detectable in bones after decomposition with drug regiments ranging from one dose to chronic exposure (6-17). A forensic case involving human skeletal remains found in a riverine environment included toxicology testing on bones,

which revealed the presence of diazepam and nordiazepam (18). This case also demonstrates the stability of compounds of interest in the bone matrix, despite environmental changes. Cases in which human skeletal remains were used for toxicological testing are otherwise very limited.

### 1.3 Cocaine

Cocaine, also known as methylbenzoylecgonine, is an alkaloid psychotropic drug derived from the South American Andes *Erythroxylon coca* plant. The Drug Enforcement Administration's Controlled Substances Act classifies cocaine as Schedule II, meaning it has high potential for abuse, but also some medicinal uses (4). It is commonly administered as a topical anesthetic for ear, nose, and throat surgery. Cocaine has the ability to block reuptake of dopamine, neurotransmitters, norepinephrine and serotonin in the brain. Desired effects of cocaine include euphoria, sexual excitement, and self-confidence whereas negative effects range from paranoia to hallucinations. Symptoms of chronic cocaine use include central nervous system stimulation, psychosis, respiratory dysfunction, rhinitis and more (4).

A 2013 National Survey on Drug Use and Health reported that there were 1.5 million persons aged 12 or older who were current users of cocaine (19). The number of persons who had cocaine dependence or abuse in 2013 is 0.3% of the population or 855,000 individuals. Though this survey shows a decrease in percentage of youths abusing cocaine between 2002 and 2013, it also shows that youths consider cocaine easier to obtain than in previous years (19).

After consumption, cocaine is rapidly distributed throughout the bloodstream, with about 90% of the compound binding to plasma proteins (20). Cocaine is primarily metabolized to benzoylecgonine via spontaneous hydrolysis at alkaline pH and ecgonine methyl ester via enzymatic hydrolysis by pseudocholinersterase. Both metabolites can also be derived via liver carboxylesterases (4).

Cocaine is the perfect candidate to consider testing for in alternative matrices such as hair, nails and bone because it is commonly abused in a chronic regiment. Because users tend to abuse this drug chronically, it has more time to become incorporated into the cell growth associated with hair and bone remodeling. Also, cocaine is very unstable in soft tissues and biological fluids. It has a half-life range of 0.5-1.5 hours in plasma (20). Cocaine and benzoylecgonine have been shown to degrade over time in blood samples at room temperature, and as much as 37% loss in urine samples stored at -20$^{o}$C for one year (21).

### 1.3.1 Testing for Cocaine in Hair

Few studies have been conducted concerning how long cocaine and its metabolites can be detected in hard tissues, such as hair, after consumption. Studies conducted using animal test subjects have shown that cocaine is quickly incorporated into hair after initial intake of the drug, and disappearance from hair is dose dependant (22). There is evidence that cocaine can remain very stable in hair after long periods of times. For example, cocaine has been detected in an analysis of hair of ancient remains of a Peruvian mummy (4). Another study has compared the concentration of cocaine in hair

before and after thermal straightening and found that while cocaine itself is degraded, metabolite concentration increases (23).

### 1.3.2 Testing for cocaine in Bone

There is a distinct lack of research studying cocaine and metabolites in skeletal remains. It has not been investigated whether cocaine, an otherwise very unstable compound, is still detectable in bones after decomposition or even immediately after death. This represents a large gap in the available research. It is important for future forensic cases to determine what drugs can be detected in alternative matrices in extreme cases of decomposition and potentially how long they will persist.

### 1.3.3 Toxicological Analysis tools

Hyphenated instrumentation, particularly liquid chromatography tandem mass spectrometry (LC-MS/MS) is widely used in forensic and clinical toxicology, particularly due to the need for high specificity and sensitivity (24). It is an approved confirmation method when screening for drugs of abuse in forensic laboratories as well as for quantitation (4, 25). LC/MS is a more flexible technique as it is ideal for more polar and thermolabile compounds whereas gas chromatography-mass spectrometry (GC/MS) can only analyze volatile compounds. It is amenable to diverse compounds and sample types. Additionally, it provides precise and reproducible quantitation. The drawback to LC/MS is that matrix effects from samples can severely alter results (24). Matrix effects are the enhancement or suppression of an analyte of interest due to the sample in which it is encased in. By incorporating sample clean-up methods prior to analysis and isolating the analyte of interest, matrix effects associated with complex samples can be reduced.

## 1.4 Instrument Theory

### 1.4.1 Liquid Chromatography

Liquid chromatography is a chemical separation process by which compounds dissolved in a liquid mobile phase are introduced to a stationary phase, which is in the form of an analytical column (26). The analytical column is typically a stainless steel tube packed with a solid material, which provides a surface for analyte interaction (26). Column materials range from porous silica to polymeric materials. Depending on the chemical compounds of the dissolved compound, they will have a higher affinity or greater interaction with the mobile phase or the stationary phase and elute from the analytical column accordingly.

High-performance liquid chromatography (HPLC), also known as high-pressure liquid chromatography, is a powerful separation method which utilizes very small particles in the stationary phase and very high pressure to force the mobile phase through the column (27). HPLC analytical columns use particle sizes around 2.5µM and higher. Ultra-performance liquid chromatography (UPLC) is the same technique as HPLC, except analytical columns consist of sub 2µm sized particles. Smaller particle size requires instrument operation with high pressure capabilities—typically above 6000psi. Separation efficiency is much higher in UPLC systems because smaller particle size in the analytical column allows for easier diffusion of analytes among particle surfaces.

Reverse-phase chromatography is among the most used separation modes for low-molecular-weight compounds, such as drugs of abuse (28). Reverse-phase chromatography applies a non-polar stationary phase and a relatively polar solvent. In

this case, non-polar compounds are typically eluted later than polar compounds due to a stronger retention of non-polar compounds on the non-polar stationary phase (27). Commonly encountered reverse-phase analytical columns are silica-based, such as $C_{18}$. A $C_{18}$ type bonded phase is prepared by combining silica with a reactive silane that carries an 18 carbon chain (28). $C_{18}$ columns have a wide range of application uses, from forensic to food and environmental, and are typically a good place to start when choosing analytical columns.

### 1.4.2 Mass Spectrometry

Typically, UPLC or HPLC systems are coupled to some kind of detector, such as a mass spectrometer. Mass spectrometry is a device which filters and separates mass fragments. After chromatographic separation, the sample is introduced to the mass spectrometer, which is under vacuum, where it is ionized, fragmented, and then the ions are separated based on their mass-to-charge ratio (29). The core instrumentation used for identification and quantitation of drugs and metabolites is liquid chromatography tandem mass spectrometry with electrospray ionization. Electrospray ionization (ESI) is a soft ionization technique which can fragment molecules into reproducible patterns (29). When the liquid sample reaches the ion source, a charge is applied to the droplet. Then, the drops are de-solvated by heated gases flowing into the source. The droplet is vaporized, charged and dispersed into positive or negative, depending on the polarity of the source, gaseous ions and ion/molecule clusters (29). Finally, the ions enter the sample cone.

Tandem mass spectrometers combine multiple mass filters in a chain within one instrument. After electrospray ionization, ions are focused in a tight beam into the first

quadrupole, known as Q1 or M1, which can be set in a scan mode to see all ions which pass into this quad or static mode to allow passage of a single ion per compound. The parent or precursor ion is discovered by conducting a Q1 scan. The precursor ions then enter the second quadrupole which is the collision cell. The cell is pressurized with argon gas which fragments the precursor molecule. After fragmentation, the ions move into the third quadrupole where they can be selectively filtered before being sent to the detector. The process from start to finish is known as multiple reaction monitoring (MRM). Using HPLC or UPLC and then selectively isolating and filtering ions using a triple quadrupole mass spectrometer allows for low detection limits in ppb (parts per billion) or less.

### 1.4.3 Two-Dimensional Liquid Chromatography

Multi-dimensional liquid chromatography employs the use of more than one separation column in one run (30). For example, a 2D-LC/MS/MS system will consist of two columns, or two dimensions, both of which could contain very different chemistries or separation modes. The biggest advantage of 2D chromatography is the potential to increase resolving power of analytes in a sample by incorporating a more chemically selective or higher resolution column on the second dimension (31). Traditional one-dimensional (1D) LC methods that employ a single column lack the versatility needed to analyze more complex samples. This is also due to the fact that only aqueous samples can be injected into the system. Full automation control is also possible with some multi-dimensional systems, which gives users the ability to automate the method development process (32).

The 2D-LC/MS/MS system utilized for this research incorporated a "Trap and Elute with At-Column Dilution" concept (32). This is a simple upgrade from a single dimensional system. All that is needed is an extra pump, valve, and a trapping column (32). The second pump is utilized for the at-column dilution concept. The dilution is performed by combining one stream connected to the injector port, the loading stream, and a dilutor stream to a low volume mixer. The flow rates of each stream are set to produce a desired dilution ratio of less than 5% (32). The diluted sample then flows onto the trap column, the first dimension, where all analytes of interest are captured. The dilution of the sample is necessary to prevent breakthrough of the analytes. The trap column is packed with large particles, greater than 10 µm. The capture of analytes depends on the chemical makeup of the compounds themselves, the chemistry of the trapping column, additives during the loading phase, and optimized flow rates. Large sample injections are possible using a 2D system, up to 1mL, because of the large particle size of the trap column and the pressure capacity of UPLC pumps. On a 1D system, only very small volumes can be directly injected without adversely affecting the chromatography, especially if the sample is in any percentage of organic solvent (33). Samples injected into a one dimensional system must be aqueous based, since high percentages of organic solvent will prevent retention of analytes onto the analytical column because a compound dissolved in an organic solvent will have more interaction with the solvent than the stationary phase. This is a concept called breakthrough. The at-column dilution concept allows the user to inject any solvent, from aqueous to organic, as well as larger volumes. For example, if a sample dissolved in 100% acetonitrile was

injected, it would first be diluted with water to less than 5%, which is a low enough percentage to prevent breakthrough. After the compounds of interest are trapped on the first dimension, the trap column is re-positioned into the elution stream where a backflush elution is performed (32). The backflush elution will flow into the higher resolution analytical column second dimension before flowing to the detector of choice.

1.4.4 Comparison of 1D and 2D sample prep methods

For one dimensional chromatography, sample preparation methods typically use, at the very minimum, a solid phase extraction, solid-liquid extraction, or liquid-liquid extraction, followed by an evaporation and reconstitution step before injection into the LC-MS/MS. Researchers who have worked with more complex matrices will use a solid-liquid extraction, evaporate to dryness, reconstitute, and then use a solid phase extraction method for further clean up. One such method used to extract drugs of abuse in bones first employs sonicating the bones in 0.25M NaOH for 60 minutes, followed by a rinsing or washing step. Next, the bones were pulverized using a general purpose grinder or mortar and pestle. The ground up bone material was incubated in methanol at 50$^{o}$C for 72 hours, evaporated, and reconstituted in an aqueous solvent. The reconstituted extract underwent a solid phase extraction step, a second evaporation to dryness, and finally reconstitution in water before injection onto an LC/MS/MS (8, 10). This method takes a minimum of 74 hours to complete. Other publications have increased the incubation time to 96 hours (17). Methods in the literature used for extraction of drugs of abuse in bone use the previously mentioned method, with some additional steps such as storage at -20$^{o}$C for one hour to precipitate lipids and proteins (6-18). The fastest method for extraction of

bones in the literature utilizes a microwave assisted extraction which takes about 15 minutes (11, 13, 14). However, the extract produced from this procedure was for immunoassay screening. The authors go on to perform a 72 hours incubation of the sample before UPLC/MS/MS or GC/MS confirmation. Analysis of hair samples utilize a similar method of a solid-liquid extraction followed by a solid phase extraction procedure, except with shorter incubation times of about 4 hours (5). Clearly, analysis of hard tissues such as bone or hair requires a large amount of time and work, which would prevent implementation of such methods into current forensic laboratories. Interestingly, the authors of these methods never explain why a prolonged incubation step is necessary for analysis of harder tissues. For analysis of softer tissues than bone or hair, such as heart, liver, or kidney, the simplest method seen in the literature employs a solid-liquid extraction of the tissue, followed by evaporation to dryness, reconstitution and direct injection into the LC/MS/MS (34). The problem with this method is that the lack of further sample clean up will most likely cause ion suppression or enhancement effects. Additionally, an evaporation to dryness step will still add one or two hours to a method.

The largest hurdle to analyzing complex matrices such as hair or bone or human tissues is the time consuming aspect of the sample preparation methods as well as the extra effort required to properly clean up the sample and isolate the analyte of interest. One of the biggest advantages to using a 2D-LC/MS/MS system is that it offers much more flexibility than a 1D system. For example, it is capable of larger injection volumes and the ability to inject 100% organic solvent. The ability to inject organic solvents means that any evaporation to dryness steps can be eliminated from a sample preparation

method, which automatically reduces preparation time by hours. Evaporative loss or redissolution issues are then no longer applicable. The extra pump, valve, and columns coupled with an automation capability means that the user can test different loading conditions, elution conditions, and trapping materials all within one overnight run, approximately 18 hours, to find the best chromatography method (35). By analyzing the data, the user can figure out if the compound of interest prefers to be trapped as an ionized or neutral species, as well as what solvent it is dissolved in—water, methanol, acetonitrile, acetone—will give the best response. All of these details will assist the user in the sample preparation method development. A two-dimensional approach has the potential to produce a multi-residue chromatography method and sample preparation method which gives a greater than 90% recovery for all compounds (35).

**1.5 Research Objective**

The objective of this research is to investigate whether cocaine and its major metabolites can be detected in bone samples after a year of decomposition and a second year of storage in room temperature conditions. This research will incorporate 2D-LC/MS/MS technology with a rapid extraction method for an overall goal runtime of less than one hour.

## 2. MATERIALS AND METHODS

### 2.1 Materials

2.1.1 Instrumentation – 2D-LC/MS/MS

For the purposes of this research, an ACQUITY UPLC® was used, with a 2D configuration for "Trap and Elute" with at-column dilution. The configuration was constructed with two Binary Solvent Managers (BSM)—one set for gradient elution and one set for "At-column" dilution. The at-column dilution pump was plumbed to create two streams. The A side was set for loading the extracts from the injection loop onto a 50 µL mixer, while the B side was set at high flow rate for dilution. A re-focusing effect on a trap column follows before the molecules are re-focused a second time onto the analytical column. The final elution of the extracts flows into the mass spectrometer for detection. The 2D-LC/MS/MS setup is depicted below (Figure 1). The detector used with the chromatography system was a Xevo TQD (Waters Corporation, Milford, MA, U.S.A) mass spectrometer.

MassLynx© version 4.1 (Waters Corporation, Milford, MA, U.S.A.) software was utilized to view and analyze all chromatograms and spectra referenced throughout this research, as well as control all instrumentation. TargetLynx© version 4.1 (Waters Corporation, Milford, MA, U.S.A) software was utilized for all quantitation performed throughout. Signal intensities were evaluated by measuring the area count of the peaks produced in the chromatograms. Each sample was analyzed in three replicate injections on the LC/MS; the average of the three injections is displayed in the following figures and tables.

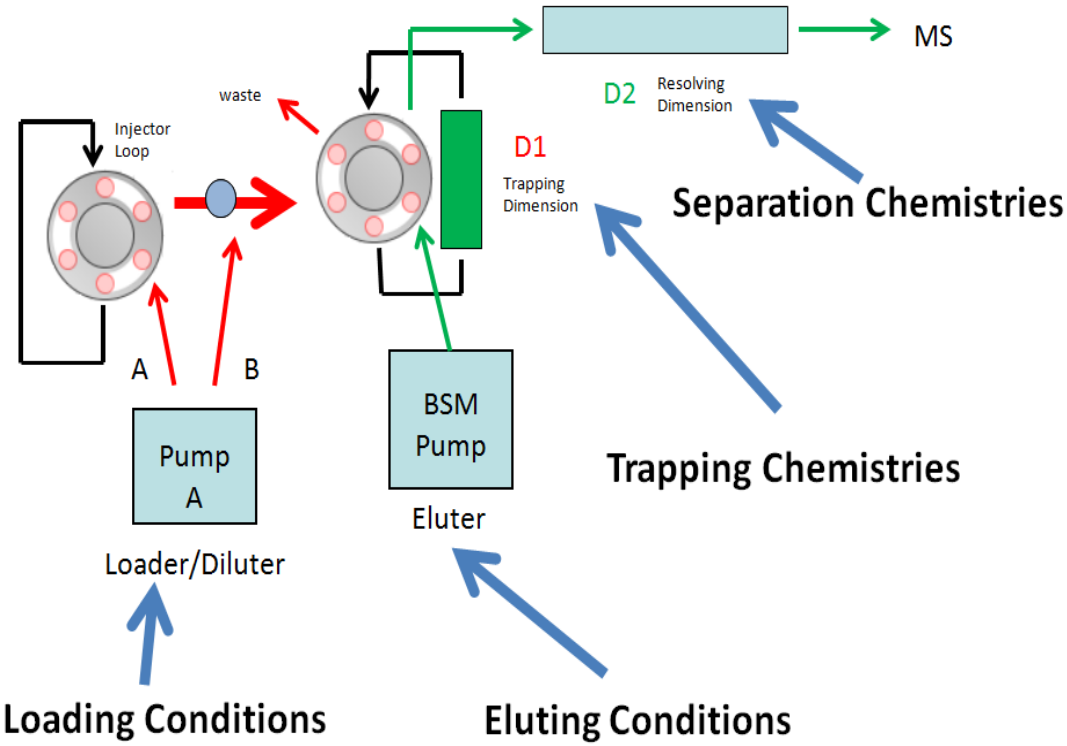



**Figure 1: Fluidic pathway of 2D-LC system**

2.1.2 Standards and Reagents

Analytical reference standards in the form of 1mg/mL solutions dissolved in methanol were obtained from Cerriliant (Round Rock, TX, U.S.A). They include cocaine, benzoylecgonine, ecgonine methyl ester, and their respective deuterated internal standards cocaine-D3, benzoylecgonine-D3, and ecgonine methyl ester-D3. All solvents utilized throughout this research, including methanol, acetonitrile, acetone, ammonium hydroxide, hydrochloric acid and formic acid, were Optima grade and purchased from

Fischer Scientific (Waltham, MA, U.S.A). All water consumed in this research for this research was Millie-Q grade water (Merck/Millipore Co, Darmstadt, Germany, EU).

## 2.2 Method development

### 2.2.1 Compound optimization

Compound optimization was performed on all standards. Each compound was prepared in a 50/50 water/methanol solution at a concentration of 10µg/mL (10ppm). The solutions were directly infused into the mass spectrometer. Precursor ions were first determined by running a scan in MS1 mode. Two product ions were then selected for each standard in MS/MS mode, while varying the collision energy values. The higher the collision energy, the more the precursor molecule is fragmented. The most intense transition was selected for quantification and the second most intense transition was selected for confirmation. The collision energy value at which the transition gave the highest signal was chosen. The MRM table for all analytes is included below.

**Table 1: MRM table for all compounds**

| Compound | Ion mode | Precursor ion | Cone | Product ion | CE |
|---|---|---|---|---|---|
| Cocaine | ESI + | 304.1 | 30 | 182.1 | 20 |
| | | | 30 | 82.1 | 30 |
| Benzoylecgonine | ESI + | 290.1 | 30 | 168.1 | 15 |
| | | | 30 | 105.1 | 30 |
| Ecgonine Methyl Ester | ESI + | 200.1 | 30 | 182.1 | 20 |
| | | | 30 | 82.1 | 25 |
| Cocaine-D3 | ESI + | 307.1 | 30 | 185.1 | 20 |
| | | | 30 | 85.1 | 30 |
| Benzoylecgonine-D3 | ESI + | 293.1 | 30 | 171.1 | 20 |
| | | | 30 | 105.1 | 30 |
| Ecgonine Methyl Ester-D3 | ESI + | 203.1 | 30 | 185.1 | 20 |
| | | | 30 | 85.1 | 25 |

An example of the compound optimization process for cocaine is depicted in the mass spectra below (Figure 2). The first chromatogram on the bottom left corner shows the compound cocaine, at mass 304da, is fully intact with no fragments at the lowest collision energy (CE) value. Then, as the collision energy increases, the product ions increase in intensity while the precursor ion diminishes. The remaining mass spectrometer settings are displayed in Table 2.

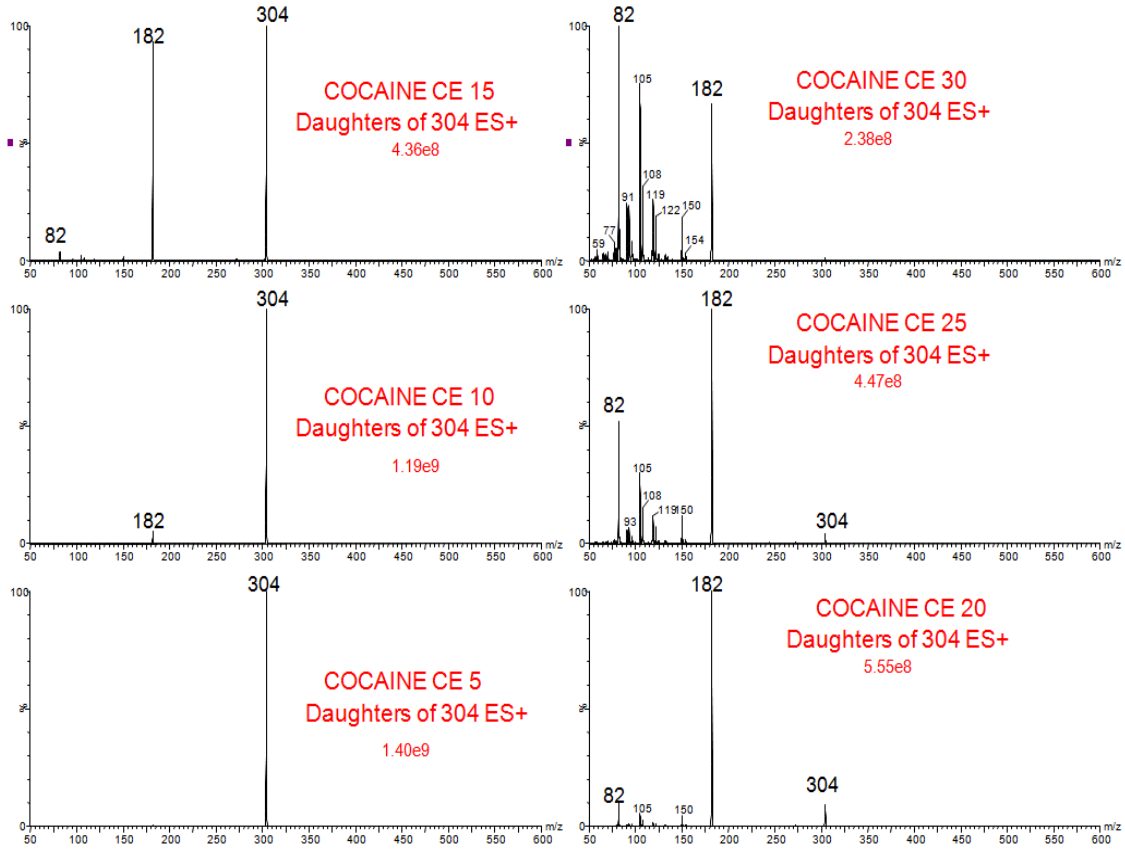



**Figure 2: Compound optimization of cocaine**

**Table 2: Mass Spectrometer settings**

| Capillary voltage | Cone voltage | Source temperature | Desolvation temperature | Desolvation gas | Cone gas |
|---|---|---|---|---|---|
| 3.0 kV | 30 V | 150$^{o}$C | 550$^{o}$C | 1100 L/hr | 50 L/hr |

2.2.2 Chromatography method development

A stock solution of cocaine, benzoylecgonine and ecgonine methyl ester was prepared at a concentration of 10µg/mL in 100% methanol. This stock solution was utilized to prepare serial dilutions of lower concentration stock solutions throughout this project. The 10µg/mL stock solution was prepared fresh each month.

The analytical separation column used for all analyses was an ACQUITY UPLC HSS T3, 2.1 x 50mm, 1.7 µm (Waters Corporation, Milford, MA, U.S.A), which is a high strength silica C18 sorbent. The sample injection volume was set at 100µL. The sample is loaded with water at 0.1mL/min and diluted at 5% with water at a flow rate of 1.9 mL/min. The mixture is loaded on the trap column at a flow rate of 2.0 mL/min. Then the compounds retained on the trap are refocused onto the analytical column during elution, which is completed with a 5 minute linear gradient from 5% of organic solvent mobile phase to 95% mobile phase. The total run time was 10 minutes.

The chromatography conditions were tested on two trapping chemistries, Oasis® HLB 40mg and 80mg trap columns (Waters Corporation, Milford, MA, U.S.A). HLB (hydrophilic-lipophilic balance) is a strong hydrophilic, high retention strength sorbent material. Additionally, loading and dilution pH was optimized by testing pH 3, 7, and 10 on the two different sized HLB trapping columns. And finally a low pH and high pH

loading was tested for each combination. An example of the chromatography evaluation scheme is pictured in Figure 3.

A 10 ng/mL stock solution was prepared in water, methanol, and Acetonitrile and run on an automated process testing different parameters in a single overnight run. Elution consisted of two mobile phases, A and B. Mobile phase A consisted of water with 0.5% formic acid and mobile phase B consisted of Acetonitrile with 0.5% formic acid. It was determined that all analytes gave the best signal intensity with a pH 10 loading and dilution on the 80mg HLB trap column.

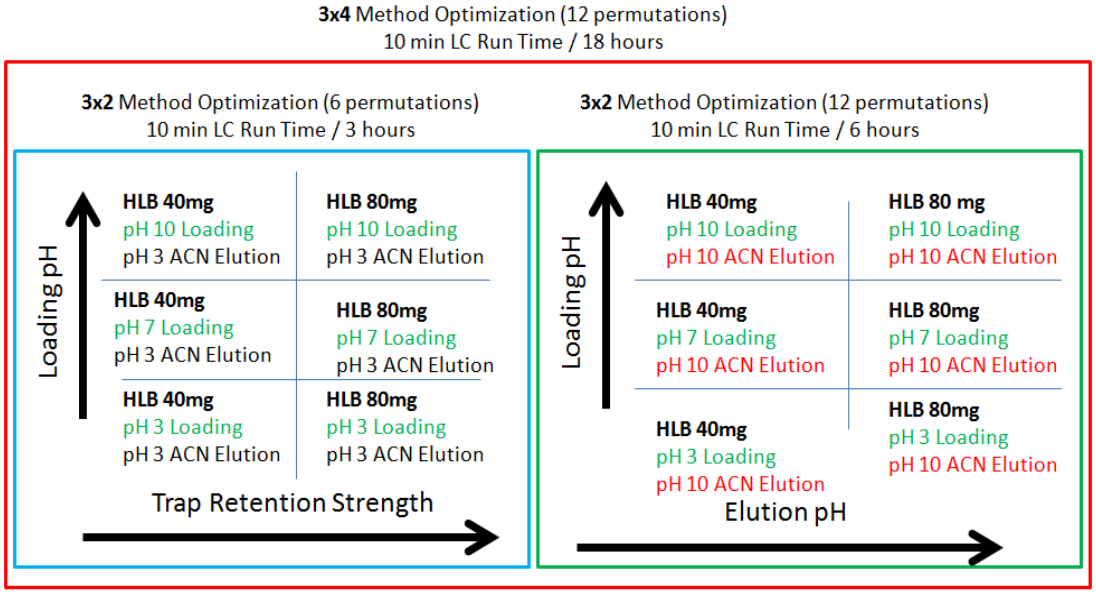



**Figure 3: Methods tested for optimization of method**

Figure 4 depicts three different methods for cocaine. All three methods utilize an 80mg HLB trapping column and low pH acetonitrile elution. The pH of the loading is varied in each column. There is an entire order of magnitude of difference in signal

intensity from the water standard to the methanol and organic standards, indicating that cocaine prefers to be dissolved in organic as opposed to aqueous. There is slight peak broadening in the standards dissolved in acetonitrile, but otherwise all methods seem to work well for cocaine.

Figure 5 depicts a similar situation to cocaine in that all methods produce Gaussian peak shapes and good intensities. However, when benzoylecgonine is dissolved in acetonitrile, there is a very apparent peak broadening. This effect could be fixed with a higher dilution factor before the first dimension, but methanol and water produced sharp peaks so no further action was taken for the acetonitrile standard. It is interesting to note in this set of chromatograms that the water standards actually produced higher signal intensities than the methanol standards, which indicates that benzoylecgonine is a more water-soluble compound. This indicated further action required in the extraction optimization discussed later.

Finally, in Figure 6, there was only one method which produced Gaussian peak shapes and good intensities. Ecgonine methyl ester needs to be loaded at a high pH in order to see any signal whatsoever. Without the ability to evaluate many chromatography methods in a short time period, it would have been much more difficult to figure out why ecgonine methyl ester was not being detected in earlier methods.

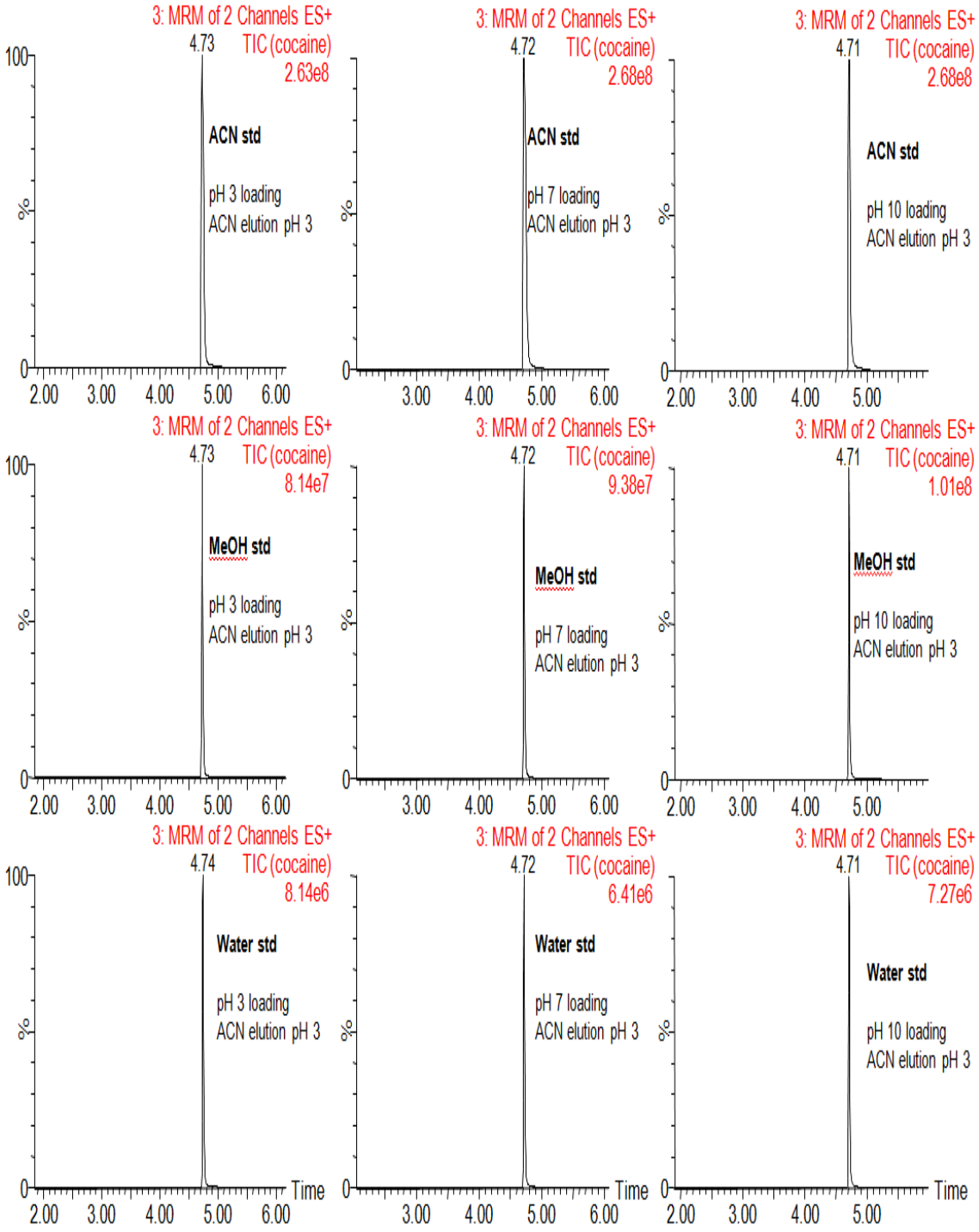



**Figure 4: Method optimization results for Cocaine**

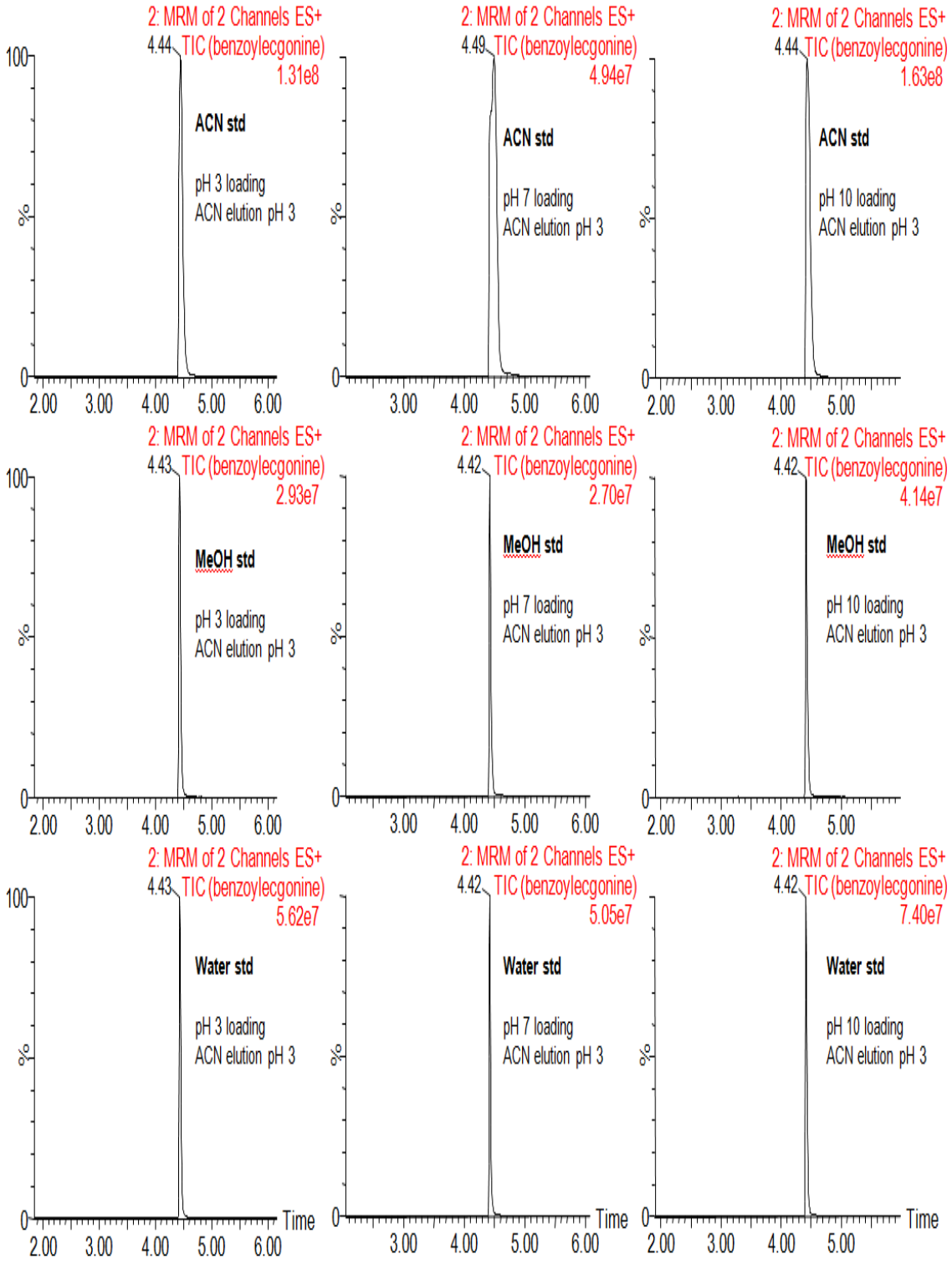



**Figure 5: Method optimization results for benzoylecgonine**

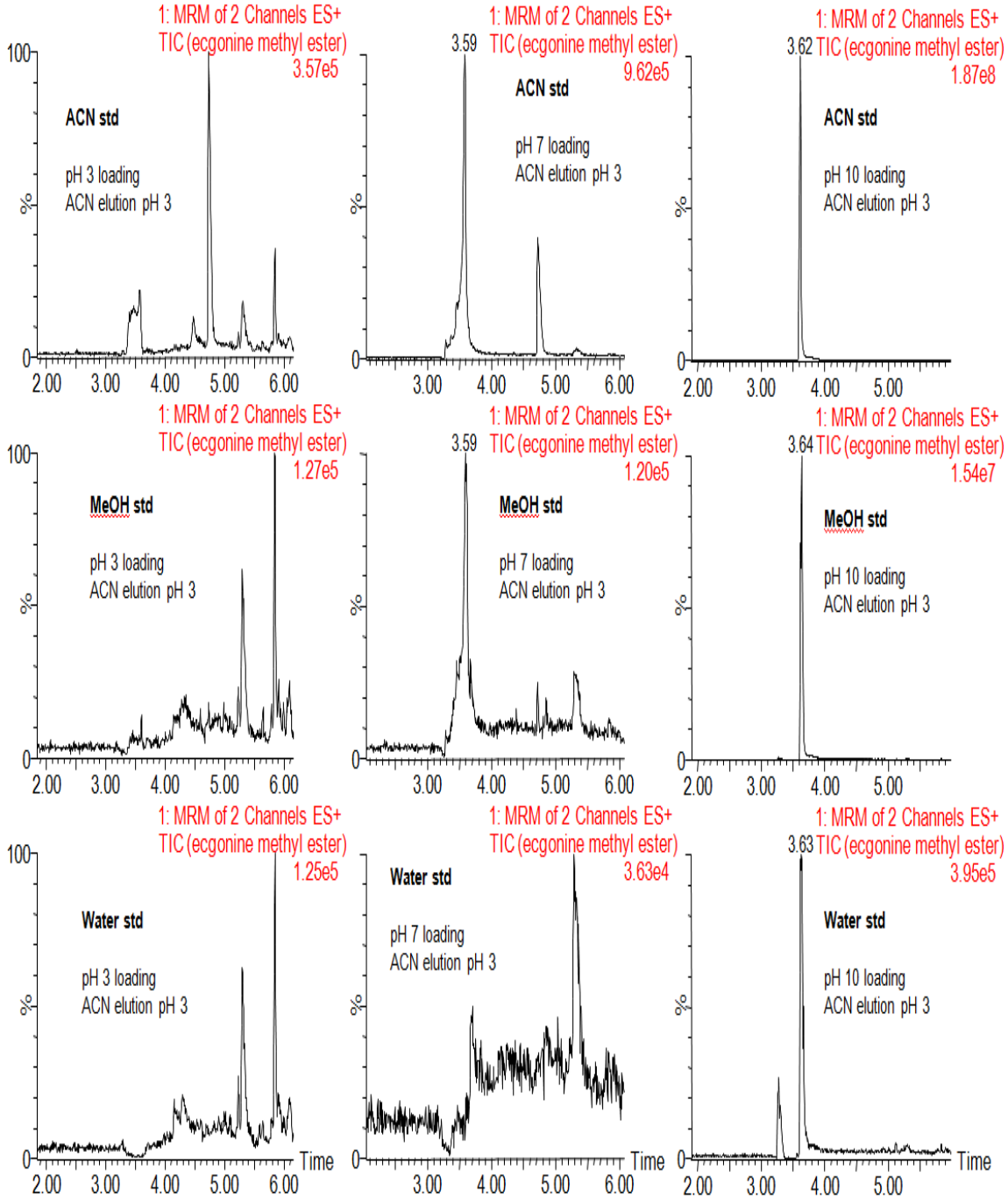



**Figure 6: Method optimization results for ecgonine methyl ester**

2.2.3 Solid phase extraction optimization

In order to develop a sample preparation method, it was necessary to choose a solid phase extraction sorbent. The pKa of each compound was referenced in preparation for this decision. Cocaine, benzoylecgonine, and ecgonine methyl ester have pKa's of 8.6, 10.82, and 9.57 respectively. All compounds are basic in nature, therefore an Oasis® MCX solid phase extraction barrel (Waters Corporation, Milford, MA, U.S.A) was chosen for testing, as it is a mixed mode sorbent consisting of a strong cation exchange portion meant to retain ionized basic compounds and a reverse phase portion meant to retain acids and neutrals. For preliminary method development, two sorbent packing sizes of this solid phase extraction sorbent were compared. The mix of compounds was prepared at a concentration of 1ng/mL (1 ppb) in methanol, Acetonitrile, acetone, 50/50 methanol and Acetonitrile, 95/5 acetonitrile and ethyl acetate, and 95/5 acetonitrile and Methyl tertial butyl ether—all with 5% ammonium hydroxide added. 2mL of each mix was dissolved in 50mL of Millipore water for loading on the 60mg MCX cartridge and 100mL of water for the 150mg MCX cartridge. The mixture of compounds, dissolved in 100% organic solvent, was diluted in Millipore water at a concentration of less than 5% so as to prevent breakthrough of the compounds on the solid phase extraction sorbent. The MCX cartridges were treated with two washes and then elution. Wash 1 was 0.1N hydrochloric acid in water, the low pH of which would ionize the basic compounds retained on the sorbent so they could be captured by the ion exchange portion. The second wash was methanol with 5% formic acid which eluted the acids and neutrals from the reverse phase portion of the sorbent, while leaving the ionized basic compounds on

the ion exchange portion. Elution was completed with the corresponding solutions mentioned above which range in polarity. For example, the mixture of compounds dissolved in methanol was also eluted from the MCX cartridge with methanol. The high pH of the elution solvent neutralized the compounds on the ion exchange sorbent and allowed them to be eluted from the sorbent. The second wash was also collected and analyzed to determine if breakthrough occurred at this step. It was determined that the 150mg MCX cartridge, using methanol as the elution solvent gave the best recoveries for all three compounds overall so it was chosen for the final method. Additionally, the second wash proved to have negligible breakthrough of all compounds.

**Table 3: Area counts of MCX 60mg evaluation**

| MCX 60mg | Cocaine | Benzoylecgonine | Ecgonine Methyl Ester |
|---|---|---|---|
| Wash 2 | 5497 | 483 | 747 |
| MeOH + 5% NH4OH | 25808 | 2199 | 5515 |
| Wash 2 | 2471 | 99 | 559 |
| ACN + 5% NH4OH | 34316 | 813 | 3187 |
| Wash 2 | 3128 | 41 | 189 |
| Acetone + 5% NH4OH | 18699 | 52 | 8 |
| Wash 2 | 3451 | 194 | 569 |
| 50/50 MeOH/CAN + 5% NH4OH | 31471 | 1529 | 1371 |
| Wash 2 | 3679 | 73 | 566 |
| 95/5 ACN/ Ethyl acetate | 33225 | 388 | 1243 |
| Wash 2 | 21 | 36 | 468 |
| 95/5 ACN/MBTE + 5% NH4OH | 46281 | 699 | 1013 |

**Table 4: Area count comparison of MCX 150mg evaluation**

| MCX 150mg | Cocaine | Benzoylecgonine | Ecgonine Methyl Ester |
|---|---|---|---|
| Wash 2 | 28 | 391 | 98 |
| MeOH + 5% NH4OH | 29915 | 10978 | 10366 |
| Wash 2 | 12 | 181 | 200 |
| ACN + 5% NH4OH | 36711 | 3192 | 5833 |
| Wash 2 | 16 | 68 | 231 |
| Acetone + 5% NH4OH | 24350 | 133 | 17 |
| Wash 2 | 14 | 243 | 249 |
| 50/50 MeOH/CAN + 5% NH4OH | 35306 | 8130 | 4652 |
| Wash 2 | 64 | 77 | 284 |
| 95/5 ACN/ Ethyl acetate | 39660 | 1138 | 3147 |
| Wash 2 | 22 | 48 | 375 |
| 95/5 ACN/MBTE + 5% NH4OH | 46354 | 896 | 3147 |

## 2.3 Bone extraction

### 2.3.1 Homogenization and solid-liquid extraction

The next step was to develop a method for extraction of cocaine and metabolites from the bone tissue. A Precellys Evolution homogenizer (Bertin Technologies, Montigny-le-Bretonneux, France, EU) was utilized for homogenization of the bone samples. The homogenization is performed by loading the Precellys with 15mL tubes containing 4mm ceramic ball bearings (Bertin Technologies, Montigny-le-Bretonneux, France, EU), which are then pressurized and shaken at speed of 5000 RPM for periods of 90 seconds at a time. In order to choose which type of ball bearings to use, one teaspoon of stainless steel ball bearings were placed in one tube and four 4mm ceramic ball bearings placed in a second tube, each containing about 0.5g of bone material and 4mL of

methanol. It was determined that it required 3 rounds of shaking for 90 seconds at 5000 RPM for both the ceramic and stainless steel ball bearings to completely pulverize the bone into a fine powder. Both types of ball bearings performed the same function in the same amount of time; however it was unclear as to whether the compounds might bind to the ball bearings or cause any kind of suppression effect. To that end, one teaspoon of new stainless steel ball bearings was placed in a tube and four new ceramic ball bearings was placed in another as well as one teaspoon of stainless steel ball bearings and four ceramic ball bearings that had been used and cleaned previously were placed in two additional tubes. In each tube, 4mL of a 1 ng/mL (1ppb) standard mix of compounds was added and shaken in the Precellys, and then run through the previously developed solid phase extraction method. Results of this experiment showed that the mix that had been treated with the new and old stainless steel ball bearings produced almost half of the signal intensity that the ceramic ball bearing treated samples did. A comparison between old and used ceramic ball bearings showed no effect, which indicated that the cleaning method utilized in between each use was efficient. In conclusion, the ceramic ball bearings were chosen for completion of all further bone extractions in this research (See Figure 7).

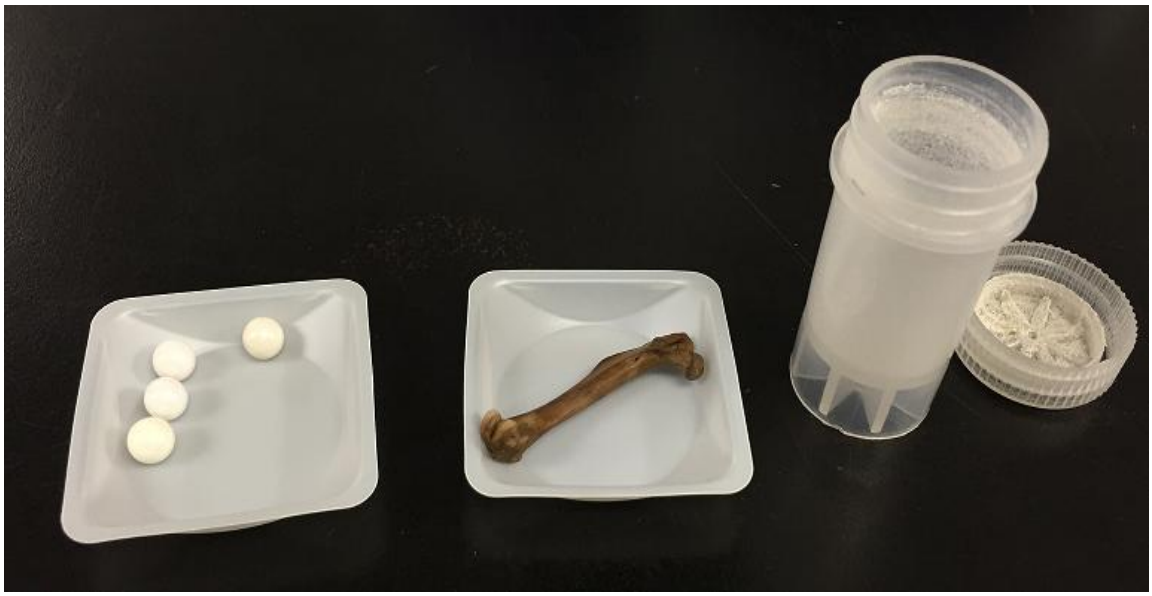


**Figure 7: Rat femur bone with ceramic ball bearings and 15mL tube used for homogenization**

After homogenization, it was necessary to choose the best extraction solvent that would give the highest recovery of the compounds of interest from bone. Millie-Q water, methanol, acetonitrile, and acetone were evaluated, each at a different pH. In each 15mL tube, 0.5 mL of bone material was added and 4mL of a 1 ng/mL (1ppb) mix of compounds was added in water, methanol, acetonitrile, or acetone. pH adjustment was achieved by addition of hydrochloric acid (for pH 1), ammonium hydroxide (for pH10), and no addition (for pH 7). After homogenization, the 15mL tubes were centrifuged at 4000 RPM for 5 minutes. The supernatant was placed in the spin tube and centrifuged a second time at 4000 RMP for 5 minutes. The final extract was then diluted in 100mL of Millie-Q water and underwent solid phase extraction via the method previously described. Results indicated that a methanol extraction at pH 7, with no additives, gave the best intensity for cocaine. However, the intensities of benzoylecgonine and ecgonine

methyl ester were relatively low. The water extraction at pH 7 gave higher intensities for benzoylecgonine and ecgonine methyl ester, but not for cocaine (See Table 5). Therefore, it was concluded that two sequential extractions were necessary for optimal extraction efficiency. This was also indicative in the chromatography evaluation where benzoylecgonine gave higher intensities when it was dissolved in water.

**Table 5. Bone extracts – first trial**

| | Cocaine | Benzoylecgonine | Ecgonine Methyl Ester |
|---|---|---|---|
| Water Bone Spike ng/mL – pH 10 | 2555 | 2241 | 694 |
| Water Bone Spike 1ng/mL – pH 7 | 27886 | 12600 | 9012 |
| Water Bone Spike 1ng/mL – pH 3 | 18010 | 5685 | 6445 |
| MeOH Bone Spike 1 ng/mL – pH 10 | 23564 | 3245 | 5852 |
| MeOH Bone Spike 1 ng/mL – pH 7 | 36409 | 6243 | 7496 |
| MeOH Bone Spike 1 ng/mL – pH 3 | 24828 | 2824 | 5256 |
| ACN Bone Spike 1 ng/mL – pH 10 | 24810 | 1243 | 5716 |
| ACN Bone Spike 1 ng/mL – pH 7 | 317 | 34 | 72 |
| ACN Bone Spike 1 ng/mL – pH 3 | 30069 | 2588 | 9333 |
| Acetone Bone Spike 1 ng/mL – pH 10 | 3249 | 89 | 32 |
| Acetone Bone Spike 1 ng/mL – pH 7 | 36350 | 355 | 3098 |
| Acetone Bone Spike 1 ng/mL – pH 3 | 27454 | 2529 | 5677 |

2.3.2 Wash step optimization

Due to the complexity of bone as a matrix, it was necessary to evaluate whether additional wash steps were required in the solid phase extraction protocol. To that end, eight bone extractions and eight water extractions were compared—both spiked at 1 ng/mL. Each set of water and bone had a different wash sequence. The first wash was always 0.1N HCl in water. The second wash varied in each set. Both single washes and

sequential washes were added to the protocol before the final elution was collected. The results of the wash evaluation are presented in Table 6.

**Table 6. Final elutions after different wash sequences. All washes have 5% formic acid additive; elution is MeOH + 5% NH4OH**

| | Final elution-water extraction | | | Final elution-bone extraction | | |
|---|---|---|---|---|---|---|
| Wash Sequence | Cocaine | BZE | EME | Cocaine | BZE | EME |
| Un-extracted std | 55861 | 32260 | 12289 | | | |
| Wash 2: 100% IPA | 44184 | 30767 | 11180 | 211 | 7 | 5 |
| Wash 2: 100% MeOH | 42533 | 28756 | 14500 | 7979 | 6996 | 3246 |
| Wash 2: 100% ACN | 41835 | 28448 | 14737 | 1708 | 1062 | 2350 |
| Wash 2: 100% ACE | 9231 | 4476 | 130 | 1251 | 832 | 441 |
| Wash 2: 50/50 Water/IPA | 41146 | 25585 | 12735 | 7986 | 1888 | 2383 |
| Wash 2: 50/50 Water/IPA Wash 3: 50/50 Water/MeOH | 37541 | 22870 | 12967 | 12296 | 8009 | 2123 |
| Wash 2: 50/50 Water/IPA Wash 3: 50/50 water/MeOH Wash 4: 50/50 Water/ACN | 46378 | 29310 | 12705 | 12674 | 5527 | 1762 |
| Wash 2: 50/50 Water/IPA Wash 3: 50/50 water/MeOH Wash 4: 50/50 Water/ACN Wash 5: 50/50 Water/ACE | 1894 | 9213 | 5805 | 13961 | 7249 | 787 |

If the objective of this research was to extract cocaine and its major metabolites from only water, the best protocol would be to add a sequential wash of 50/50 water isopropanol, 50/50 water and methanol, and 50/50 water and acetonitrile before the final elution. However, the results are very different for the bone extractions. While the recovery of cocaine does increase with more washes, the metabolites, particularly ecgonine methyl ester, decreases with every additional wash. Therefore, it was determined that the best protocol for all metabolites overall would be the 100% methanol wash only.

### 2.3.3 Extraction efficiency

In order to evaluate the efficiency of the final method, un-extracted standards, water extractions, and bone extractions were compared. The water extractions were loaded at pH 1, 7, and 10, and the final bone extracts were loaded at pH 1, 7, and 10 as well. Two different extraction schemes were compared for bone. The first was a methanol + 2% HCl extraction, followed by a water + 2% HCl extraction. The supernatants were pooled before dilution. The 100mL water pH was adjusted with either hydrochloric acid or ammonium hydroxide for pH 1 and 10 respectively. The second scheme was a methanol extraction followed by a water extraction with no additives. The supernatants were pooled, diluted, and pH adjusted before loading onto the solid phase extraction sorbent. With the exception of ecgonine methyl ester, the water extractions for cocaine and benzoylecgonine produced recoveries over 90%. There is low extraction efficiency for ecgonine methyl ester in both water and bone. When comparing the bone extractions to the water extractions, it was determined that a pH 10 load gave the best

overall recovery for all three compounds in comparison to the water extraction values. See table 7.

**Table 7. Un-extracted standard vs water extraction vs bone extraction at different loading pH**

| | Cocaine | Benzoylecgonine | Ecgonine Methyl Ester |
|---|---|---|---|
| Un-extracted std – 1 ng/mL MeOH | 138434 | 58633 | 26338 |
| Water extraction – 1 ng/mL spike | | | |
| pH 1 load | 132113 | 57354 | 11875 |
| pH 7 load | 125769 | 55301 | 11809 |
| pH 10 load | 112949 | 42164 | 11725 |
| Bone extraction – 1 ng/mL spike | | | |
| pH 1 load - MeOH + water + 2% HCl | 75568 | 28767 | 3971 |
| pH 1 load – MeOH + water – no additives | 80143 | 31784 | 9701 |
| pH 7 load - MeOH + water + 2% HCl | 62008 | 24017 | 4880 |
| pH 7 load – MeOH + water – no additives | 78449 | 29321 | 10813 |
| pH 10 load - MeOH + water + 2% HCl | 72581 | 30894 | 6569 |
| pH 10 load – MeOH + water – no additives | 97785 | 32071 | 9758 |

It was apparent that, during method development, there was a need for a filtration step before the dilution step. The supernatants of the bones after homogenization and centrifugation still contained particles which caused the solid phase extraction to clog. Therefore several filters were evaluated to ensure that the compounds of interest would not bind or be retained on the filters and prevent optimal recovery. A centrifuge spin tube

containing a 0.45µm PTFE filter was chosen. After centrifugation of the bones, the supernatant was poured into the spin tubes and then centrifuged again at 4000 RPM for 5 minutes. A second bone extraction was performed by adding 4mL of Millipore water to the already homogenized bone in the 15mL tube and shaking the mixture in the Precellys for an additional 90 seconds. The water extract was centrifuged again and filtered with spin tube. The water and methanol extracts were pooled together in a 15mL centrifuge tube before undergoing a centrifugation step one final time. The resulting solution was diluted in 100mL of water before solid phase extraction. The final extraction method is displayed below in figure 8.

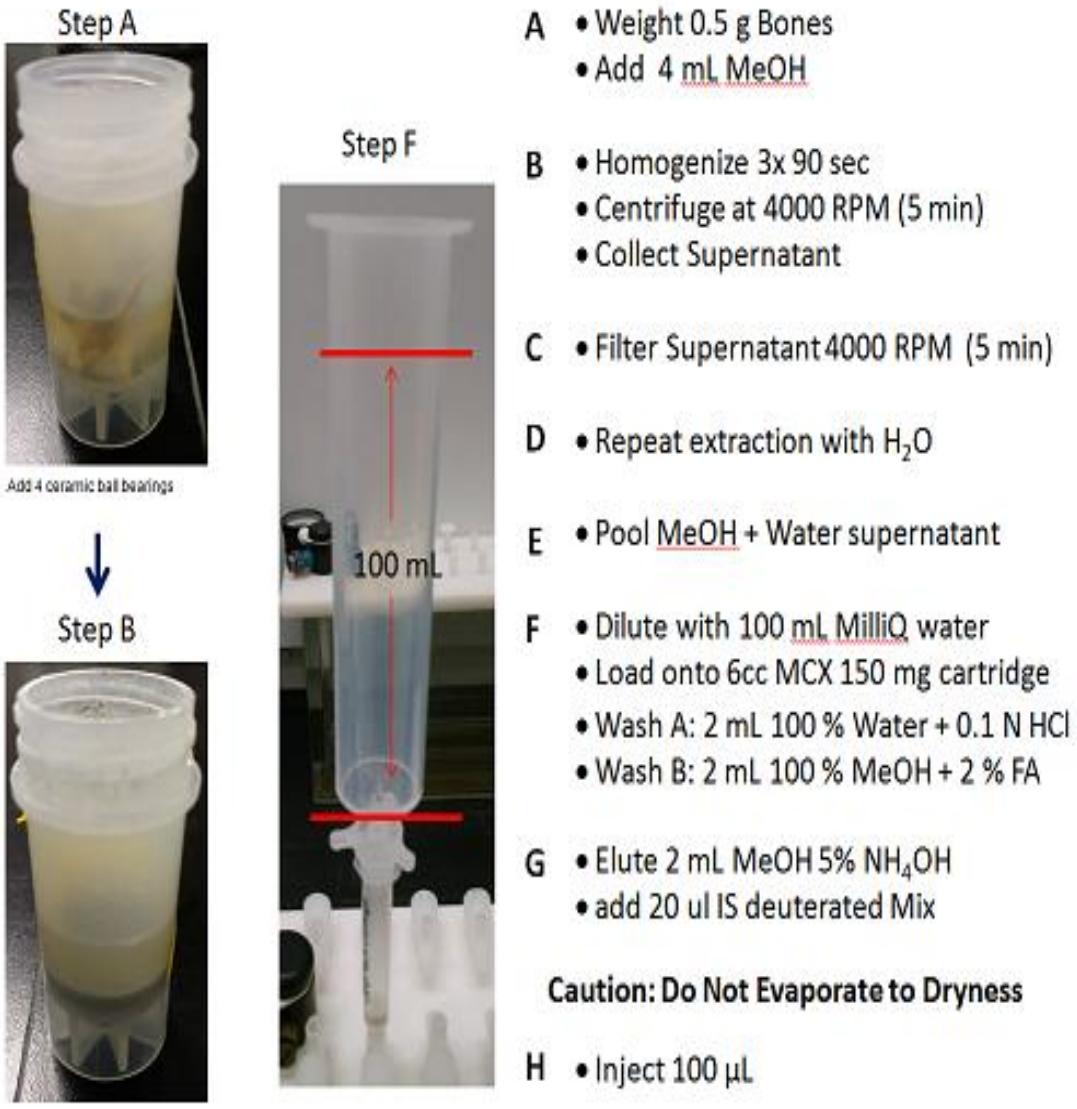



**Figure 8: Final extraction protocol**

2.4 Long-term Cocaine exposure to rat specimens

All rat specimens used for this study fell under an Institutional Animal Care and Use Committee (IACUC) protocol. The rodents underwent 10-12 weeks chronic intravenous self-administration of cocaine. This was followed by a six-week period of abstinence, followed again by a three-week period of cocaine self-administration before

being euthanized. Average daily dosages for each rat fell within a range of 13-19 mg/kg. 14 cocaine positive rats were placed outside and above ground in a gated facility for a period of 12 months. All recoverable skeletal samples were collected, cleaned, and stored at room temperature for an additional year before testing. Drug free control rat bones were also acquired by placing drug-free rats outdoors, above ground in the Boston University Forensic Anthropology Outdoor Facility (Holliston, MA, U.S.A), until full decomposition occurred. Bone specimens were grouped by bone type and each sample averaged about 0.5g. 21 femora, 20 tibio-fibula, 19 humeri + ulna combinations, and 17 innominate bones were prepared via the final extraction method described above. Additionally, one humerus and ulna combination, two ulna and two radii combination, 0.5g of vertebrae and one tibio-fibula taken from a rat immediately after being euthanized was analyzed for comparison. After the one year period of decomposition, all recovered bones were washed with water and stored in plastic containers at room temperature. The bones retrieved immediately after death of the rodent specimen were cleaned using a beetle colony to assist in consumption of the remaining soft tissue.

## 3. RESULTS

### 3.1 Calibration model

A calibration curve ranging from 0.05 ng/mL to 10 ng/mL was run in both water and bone on the same day as the final extractions to assist in quantitation. Calibrators were prepared by making a set of concentrated solutions in methanol, including 0.05 ng/mL, 0.1 ng/mL, 0.25 ng/mL, 0.5ng/mL, 1.0 ng/mL, 2.5ng/mL, 5.0 ng/mL and 10 ng/mL. The dilution calculations are displayed in Table 8. First, three 10 mL stock solutions containing cocaine, benzoylecgonine, and ecgonine methyl ester were prepared: Stock A = 1000 ng/mL, Stock B = 100 ng/mL, and Stock C = 10 ng/mL.

**Table 8: Calibrator concentration calculations**

| | Stock solution volume (µL) | Methanol volume (mL) | Final concentration (ng/mL) |
|---|---|---|---|
| Stock A | 100 | 9.9 | 10 |
| | 50 | 9.95 | 5 |
| | 25 | 9.975 | 2.5 |
| Stock B | 100 | 9.9 | 1 |
| | 50 | 9.95 | 0.5 |
| | 25 | 9.975 | 0.25 |
| Stock C | 100 | 9.9 | 0.1 |
| | 50 | 9.95 | 0.05 |

The calibrators were used to spike 100mL water solutions as well as 0.5g bone samples. A full un-extracted curve containing only neat standards, a water extracted curve, and a bone extracted curve were analyzed on the same day as the unknown samples. Additionally, a blank and a recovery sample at a concentration of 1 ng/mL were run with the water extraction and the bone extraction. A mixture of internal standards containing cocaine-D3, benzoylecgonine-D3, and ecgonine methyl ester-D3 was post-spiked to all

final elutions at a concentration of 1 ng/mL. The recovery sample is analyzed with the curve as an unknown in order to calculate how accurate the curve is when analyzing an unknown in the same matrix. A ratio of the internal standard area count and the calibrator area count of each concentration was used to calculate the calibration curve equation. The final linear dynamic range for cocaine was 0.05ng/mL to 10ng/mL, and 0.1ng/mL to 10ng/mL for benzoylecgonine and ecgonine methyl ester. The un-extracted standard calibration curve, the water extraction calibration curve (see appendix), and the bone extraction calibration curve all show excellent linearity with $R^2$ values greater than 0.995 (See figures 9-11).

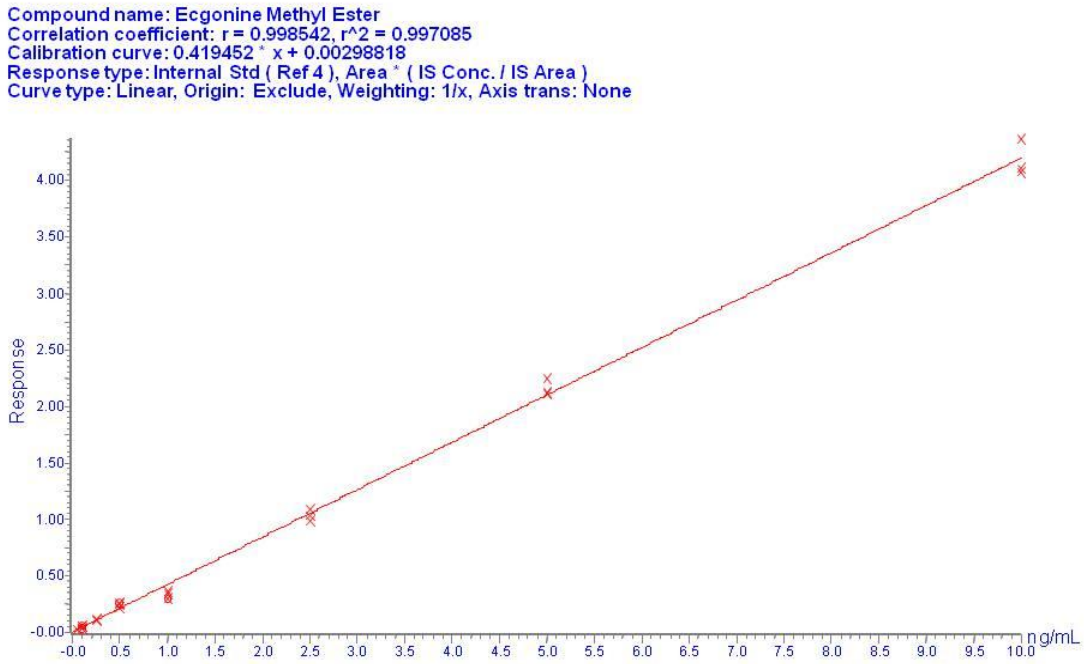



**Figure 9: Calibration curve for ecgonine methyl ester**

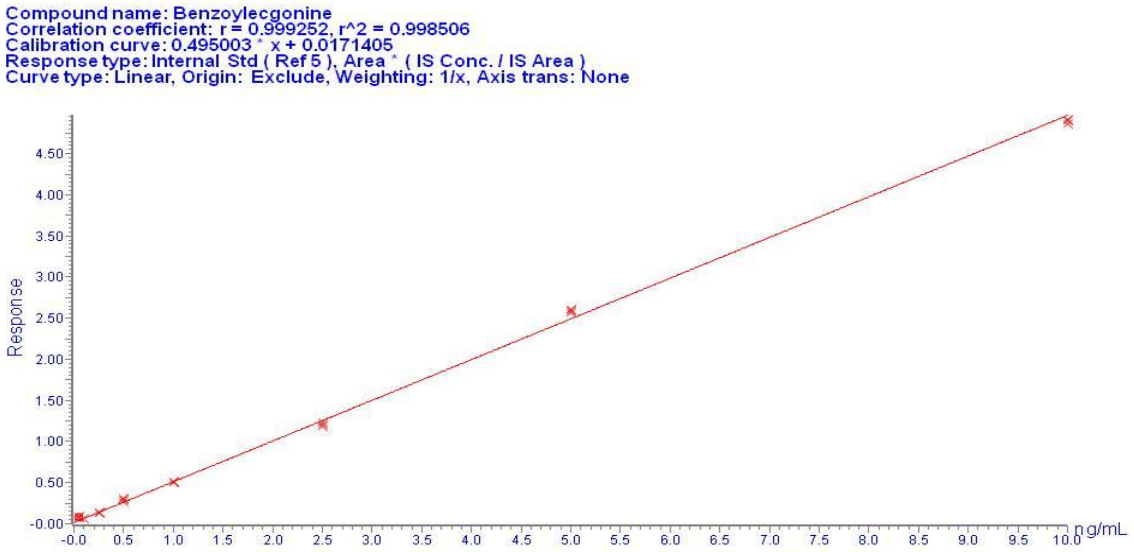



**Figure 10: Calibration curve for benzoylecgonine**

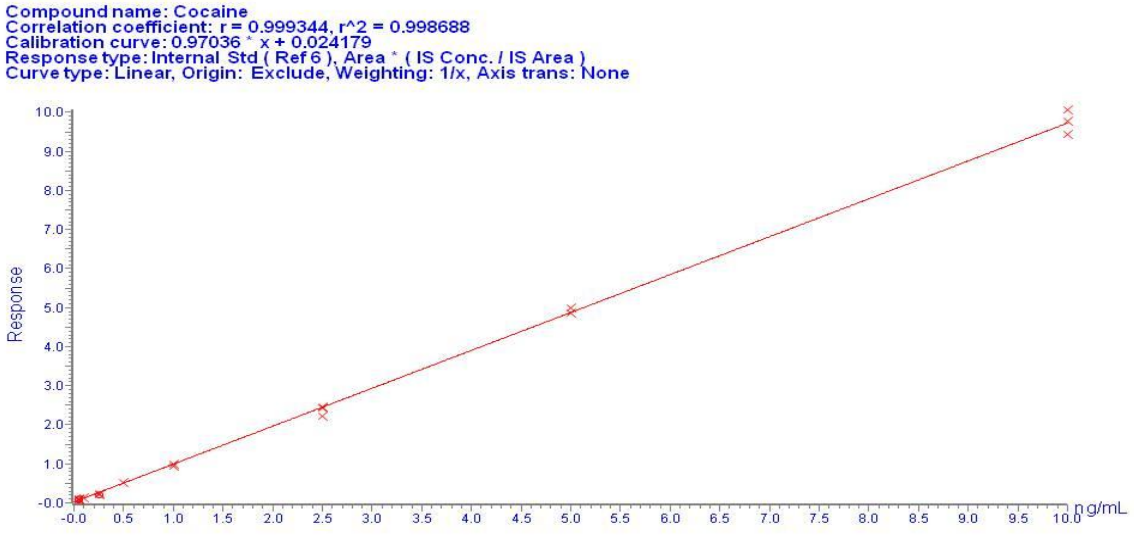



**Figure 11: Calibration curve for cocaine**

The within curve precision was calculated using the TargetLynx software. All points on all curves demonstrated less than 20% deviation. The 1 ng/mL recovery sample for ecgonine methyl ester was calculated at 88.7% accuracy in the calibration curve, 80% for benzoylecgonine, and 94.3% accurate for cocaine.

### 3.2 Comparison of un-extracted standards and extracted standards

The un-extracted calibration curve was compared to the water extraction calibration curve and the bone extraction calibration curve in order to evaluate the background noise, matrix suppression, and recovery calculations. Figure H demonstrates chromatograms of all three compounds in all three calibration curves at a concentration of 0.5 ng/mL. The chromatograms demonstrate that the un-extracted standards give a Gaussian peak shape with little to no background noise, while the water and bone extractions show minor interferences and a noisy baseline. Additionally, between all curves, the retention time of all compounds remains consistent.

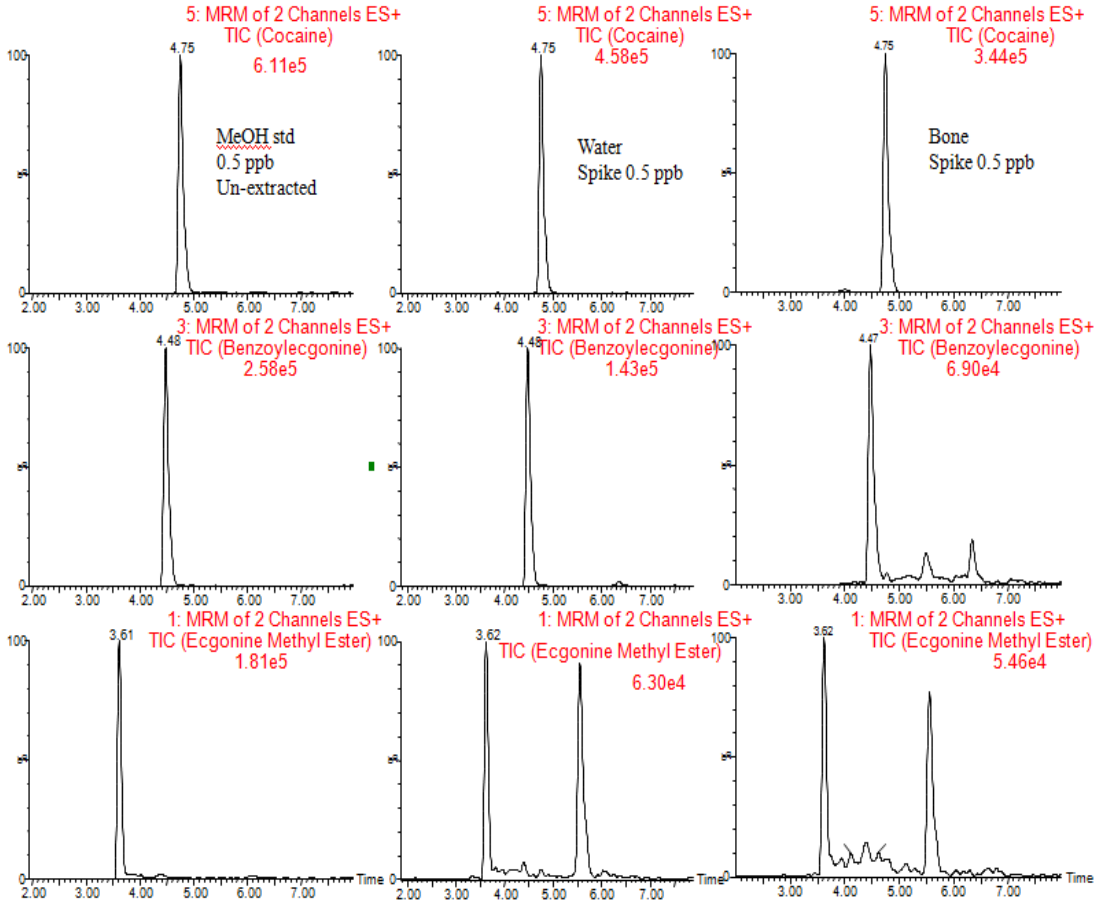



**Figure 12: Comparison of un-extracted and extracted standard**

3.2.1 Matrix suppression and recovery calculations

The following three figures demonstrate each compound with its corresponding internal standard for the neat standard and extracted standard curves (Figure …). The intensity or area count of both the compound and the internal standard is displayed as well as the suppression calculations, the ion ratios, the extracted recovery and un-extracted recovery. For ecgonine methyl ester, the 0.25ng/mL concentration displayed, as well as the internal standard at 1 ng/mL. The chromatograms for benzoylecgonine and cocaine demonstrate a 1.0ng/mL analyte concentration and the internal standard post-

spiked at 1ng/mL. The matrix suppression was calculated by comparing the intensity or area count of the internal standard of the neat standard and the area count of the post-spiked internal standard of the extracted standard. The percentage difference between the values is the calculated suppression. The suppression effect for the 100mL Millipore water enrichment process is 6.3% for ecgonine methyl ester, 8.8% for benzoylecgonine, and 9.5% for cocaine. The suppression effect increases when the bone matrix is added to the equation. The bone matrix incorporation gives a suppression effect of 8.6% for ecgonine methyl ester, 24% for benzoylecgonine, and 21% for cocaine. It is expected that bone would cause a larger amount of suppression of the compounds because it is a very complicated matrix containing many potential interferences.

Extraction recovery was calculated in two ways – based on a comparison of the area count or intensity between the neat standards and extracted standards and a comparison of the ion ratios of the un-extracted and extracted standards. The area counts of the extracted standards are compared directly to the un-extracted standard to demonstrate how effective the extraction method is for both water and bone compared to the standards used to spike them. The ion ratios are used to normalize the data and provide a more accurate recovery calculation between matrices. The ion ratios are calculated by dividing the area count of the analyte by the area count of the corresponding internal standard. The ion ratios were then used to calculate the recovery of compounds between un-extracted and extracted standards.

The water extraction recovery was calculated using the area count and the ion ratio of the water extraction and that of the un-extracted standard. In the water extraction,

ecgonine methyl ester showed a 54% recovery based on the compound's intensity, and a 57% recovery based on the ion ratio. Benzoylecgonine had a 72% recovery based on the compound's intensity, and an 80% recovery based on the ion ratio. Cocaine had an 85% recovery based on the compound's intensity and a 94% recovery based on the ion ratio. The bone extraction recovery included both an un-extracted standard recovery and an extracted standard recovery. First the analyte area count was compared to the un-extracted standard area count. Then, the ion ratio was compared to both the un-extracted standard ion ratio and the water extracted standard ion ratio, referred to as the un-extracted standard recovery and the extracted standard recovery, respectively. The extracted standard recovery was included as it is a more accurate representation of the sample preparation method's extraction efficiency between a very clean matrix and a very complicated matrix. Ecgonine methyl ester had a 39% recovery based on area count, an un-extracted standard recovery of 52% and an extracted standard recovery of 65%. Benzoylecgonine showed a 39% recovery based on area count, an un-extracted standard recovery of 52% and an extracted standard recovery of 65%. Cocaine had a 64% recovery based on area count, an un-extracted standard recovery of 81% and an extracted standard recovery of 86%. Overall, cocaine demonstrated the best recoveries overall. High recovery calculations for cocaine prove that the sample preparation method described in the previous chapter is very efficient at extracting and isolating cocaine from both a clean and very complicated matrix, though the method requires more work to increase extraction efficiencies of the metabolites overall.

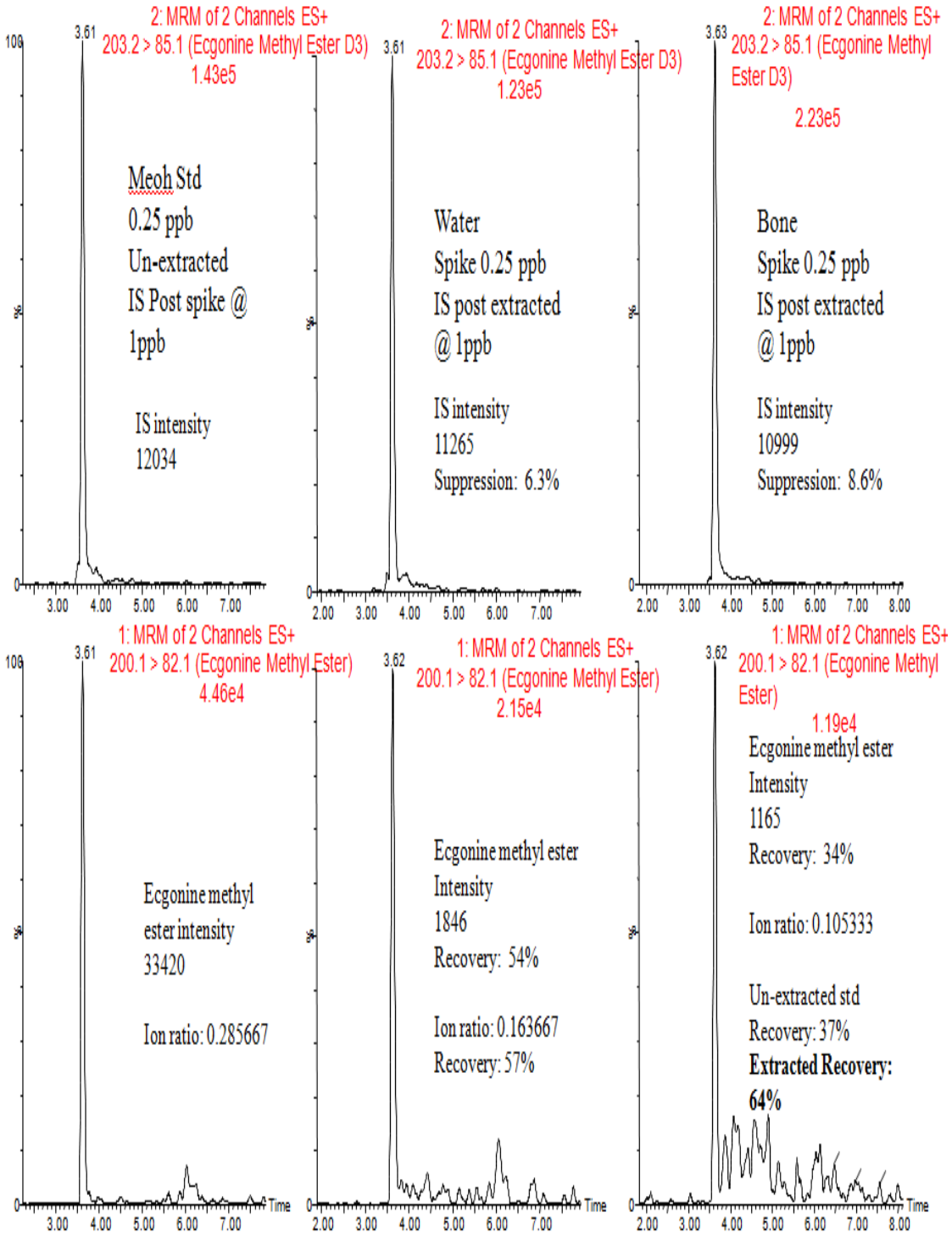



**Figure 13: Matrix effects and recovery calculations for ecgonine methyl ester**

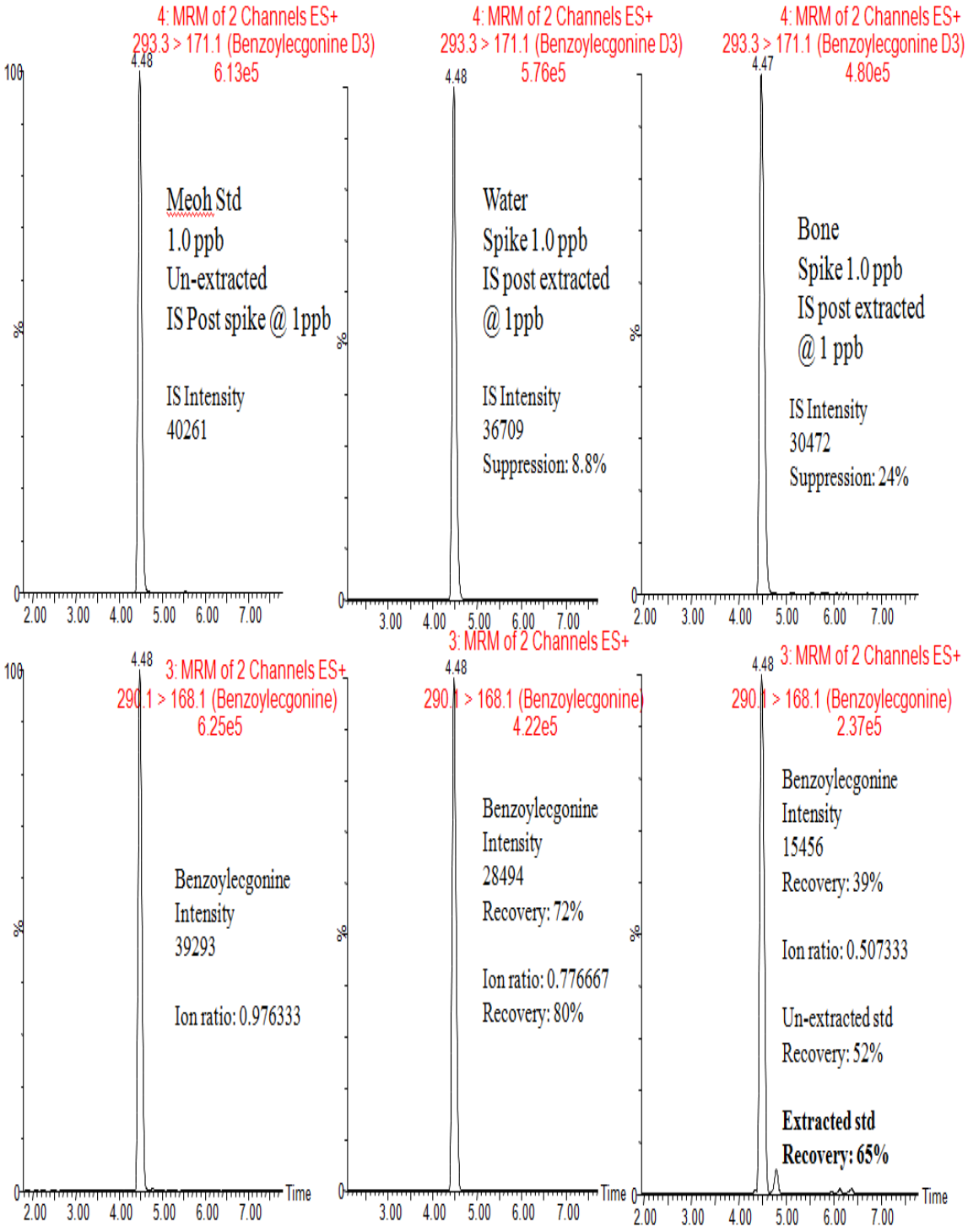



**Figure 14: Matrix effects and recovery calculations for benzoylecgonine**

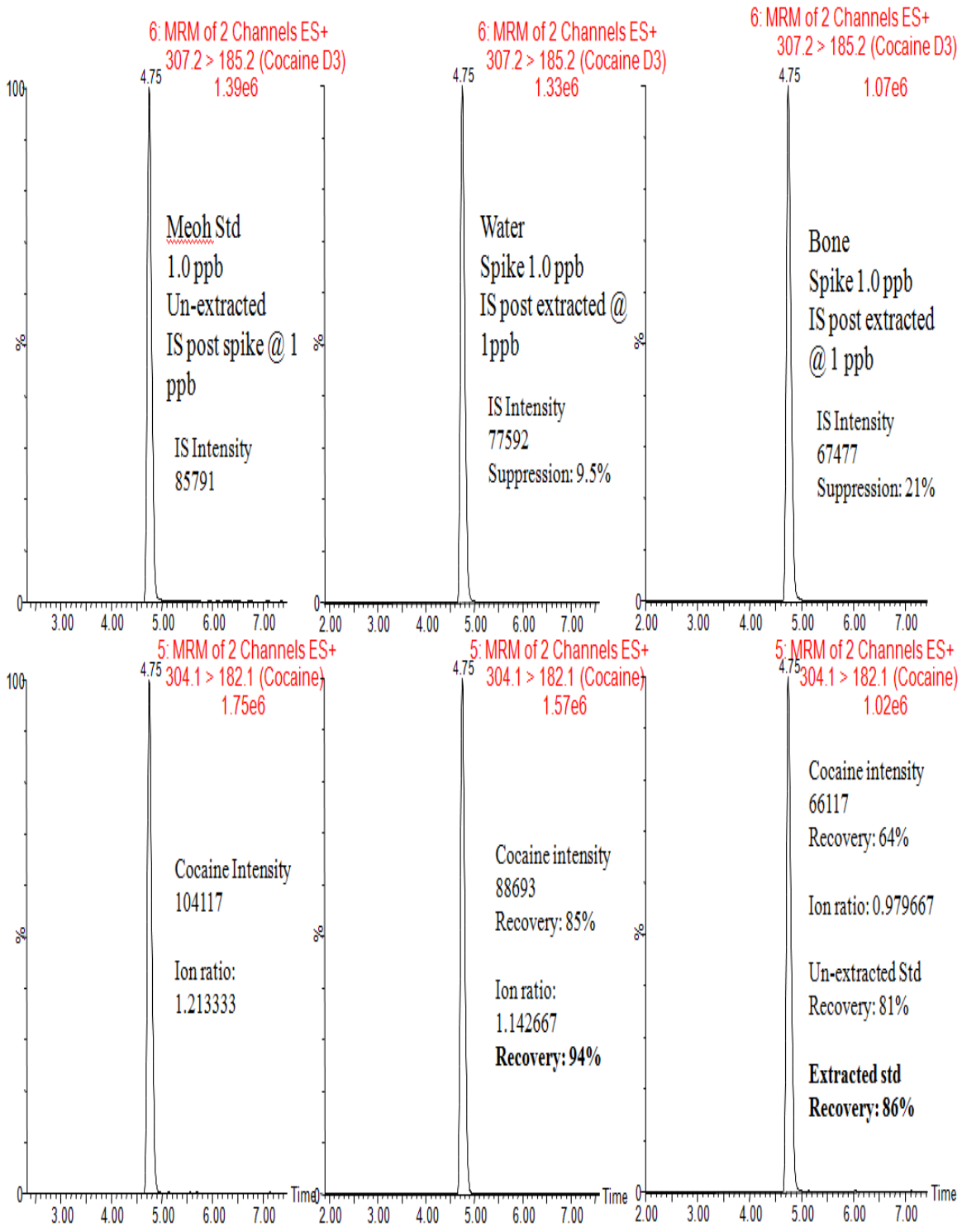



**Figure 15: Matrix effects and recovery calculations for cocaine**

### 3.3 Quantitation of unknown samples

The results of the calculated concentrations of the biological specimens are displayed in Tables 9-13, divided by bone type. Quantitation of each specimen was achieved by using the linear equations generated from the calibration curve in bone, shown above. The biological specimens include 17 innominate bones, 21 femora, 19 humerus + ulna combinations, and 20 tibio-fibulae. Each set of bones ranged from 0.5g to 1.0g of material. The final table depicts the bone specimens retrieved from a rodent carcass immediately after death.

With the exception of cocaine, the bones analyzed at Time zero ($T_0$) all show the greatest concentrations of all analytes. Ecgonine methyl ester was not detected in any bone analyzed after two years ($T_{2\ years}$). Trace level detection (TLD) indicates concentrations below 0.0001ng/g or 0.1pg/g.

**Table 9: Quantitation of compounds in innominate bone**

| | Cocaine ng/g | Benzoylecgonine ng/g | Ecgonine methyl ester ng/g |
|---|---|---|---|
| Innominate 1 | 0.007 | 0.052 | ND |
| Innominate 2 | TLD | 0.065 | ND |
| Innominate 3 | 0.052 | 0.05 | ND |
| Innominate 4 | 0.005 | TLD | ND |
| Innominate 5 | 0.001 | 0.022 | ND |
| Innominate 6 | 0.05 | 0.027 | ND |
| Innominate 7 | 0.001 | 0.033 | ND |
| Innominate 8 | 0.008 | 0.019 | ND |
| Innominate 9 | TLD | TLD | ND |
| Innominate 10 | 0.011 | 0.003 | ND |
| Innominate 11 | 0.0008 | TLD | ND |
| Innominate 12 | TLD | TLD | ND |
| Innominate 13 | TLD | 0.031 | ND |
| Innominate 14 | TLD | 0.027 | ND |
| Innominate 15 | 0.009 | 0.032 | ND |
| Innominate 16 | 0.004 | ND | ND |
| Innominate 17 | 0.0002 | TLD | ND |

**Table 10: Quantitation of compounds in Femora**

| | Cocaine ng/g | Benzoylecgonine ng/g | Ecgonine methyl ester ng/g |
|---|---|---|---|
| Femur 1 | TLD | TLD | ND |
| Femur 2 | TLD | 0.026 | ND |
| Femur 3 | TLD | 0.115 | ND |
| Femur 4 | TLD | 0.1 | ND |
| Femur 5 | TLD | 0.021 | ND |
| Femur 6 | TLD | 0.054 | ND |
| Femur 7 | TLD | TLD | ND |
| Femur 8 | 0.0002 | 0.033 | ND |
| Femur 9 | 0.04 | 0.024 | ND |
| Femur 10 | 0.12 | 0.005 | ND |
| Femur 11 | TLD | 0.013 | ND |
| Femur 12 | TLD | 0.017 | ND |
| Femur 13 | 0.008 | 0.019 | ND |
| Femur 14 | 0.027 | 0.108 | ND |
| Femur 15 | 0.013 | 0.056 | ND |
| Femur 16 | 0.028 | 0.019 | ND |
| Femur 17 | 0.021 | 0.112 | ND |
| Femur 18 | 0.024 | TLD | ND |
| Femur 19 | 0.137 | 0.05 | ND |
| Femur 20 | 0.018 | ND | ND |
| Femur 21 | 0.018 | ND | ND |

**Table 11: Quantitation of compounds in humeri and ulna**

| | Cocaine ng/g | Benzoylecgonine ng/g | Ecgonine methyl ester ng/g |
|---|---|---|---|
| Humerus + ulna 1 | 0.026 | TLD | ND |
| Humerus + ulna 2 | TLD | TLD | ND |
| Humerus + ulna 3 | TLD | TLD | ND |
| Humerus + ulna 4 | TLD | TLD | ND |
| Humerus + ulna 5 | TLD | 0.014 | ND |
| Humerus + ulna 6 | 0.019 | TLD | ND |
| Humerus + ulna 7 | 0.062 | 0.127 | ND |
| Humerus + ulna 8 | TLD | TLD | ND |
| Humerus + ulna 9 | 0.12 | 0.062 | ND |
| Humerus + ulna 10 | TLD | TLD | ND |
| Humerus + ulna 11 | 0.019 | TLD | ND |
| Humerus + ulna 12 | TLD | ND | ND |
| Humerus + ulna 13 | TLD | ND | ND |
| Humerus + ulna 14 | TLD | ND | ND |
| Humerus + ulna 15 | TLD | ND | ND |
| Humerus + ulna 16 | 0.183 | 0.029 | ND |
| Humerus + ulna 17 | 0.047 | 0.013 | ND |
| Humerus + ulna 18 | 0.008 | TLD | ND |
| Humerus + ulna 19 | 0.0005 | ND | ND |

**Table 12: Quantitation of compounds in tibio-fibulae**

| | Cocaine ng/g | Benzoylecgonine ng/g | Ecgonine methyl ester ng/g |
|---|---|---|---|
| Tibio-Fibula 1 | TLD | TLD | ND |
| Tibio-Fibula 2 | TLD | TLD | ND |
| Tibio-Fibula 3 | TLD | ND | ND |
| Tibio-Fibula 4 | TLD | 0.034 | ND |
| Tibio-Fibula 5 | TLD | 0.005 | ND |
| Tibio-Fibula 6 | TLD | TLD | ND |
| Tibio-Fibula 7 | TLD | 0.022 | ND |
| Tibio-Fibula 8 | TLD | TLD | ND |
| Tibio-Fibula 9 | TLD | ND | ND |
| Tibio-Fibula 10 | 0.0159 | TLD | ND |
| Tibio-Fibula 11 | TLD | TLD | ND |
| Tibio-Fibula 12 | TLD | 0.017 | ND |
| Tibio-Fibula 13 | TLD | TLD | ND |
| Tibio-Fibula 14 | 0.0005 | TLD | ND |
| Tibio-Fibula 15 | TLD | TLD | ND |
| Tibio-Fibula 16 | TLD | 0.017 | ND |
| Tibio-Fibula 17 | TLD | 0.020 | ND |
| Tibio-Fibula 18 | TLD | 0.026 | ND |
| Tibio-Fibula 19 | TLD | 0.010 | ND |
| Tibio-Fibula 20 | TLD | TLD | ND |

**Table 13: Quantitation of bones at $T_0$**

| | Cocaine ng/g | Benzoylecgonine ng/g | Ecgonine methyl ester ng/g |
|---|---|---|---|
| Humerus + ulna | 0.030 | 0.067 | 0.047 |
| 2 Ulna + 2 radii | 0.028 | 0.055 | 0.049 |
| Vertebrae | 0.040 | 0.165 | 0.201 |
| Tibio-fibula | 0.085 | 0.184 | 0.201 |

Representative chromatograms of the specimens are included in Figures 16-18. The remaining chromatograms for all case samples can be found in Appendix A. The left column of chromatograms show four representative spiked bone concentrations in the

calibration curve, the left column of chromatograms depicts a representative bone in varying concentrations.

The first figure gives a representation of cocaine detected in the vertebrae sample from time zero, the innominate 3, the femur 19, and the humerus + ulna 16. The concentrations were measured at 0.04ng/g, 0.052 ng/g, 0.137ng/g, and 0.183ng/g or 40pg/g, 52pg/g, and 183pg/g respectively. As demonstrated, even at concentrations as low as 0.05ng/g or 50pg/g the baseline is very low with no interferences. This is an indication that the linear dynamic range of cocaine could have been extended further into lower concentrations. Additionally, all of the peaks are Gaussian and the retention times are stable. Cocaine is clearly very stable in bone for longer periods of time.

Figure 17 represents benzoylecgonine detected in different bone types. Benzoylecgonine was detected in the vertebrae at time zero, the innominate 3, the femur 14, and the humerus + ulna 7, at concentrations of 0.165ng/g, 0.050ng/g, 0.108ng/g, and 0.127ng/g or 165pg/g, 50pg/g, 108pg/g and 127pg/g respectively. Besides the two $T_{2\ years}$ samples at concentrations 0.100ng/g or 100pg/g, the rest of the $T_{2\ years}$ samples ranged from 0.010ng/g to 0.050ng/g or 10pg/g to 50pg/g. The high concentration of benzoylecgonine in the time zero sample may indicate that benzoylecgonine has a greater chance of being detected in higher concentrations closer to the time of death. The lower concentrations may be an indication of sample degradation as well. It is also interesting to note that the baseline gets noisier as the concentration decreases. This indicates that the linear dynamic range listed for this compound was as accurate as possible. It is

interesting to note that the interferences vary from bone to bone, which means that each sample tested is different.

Finally, Figure 18 has representative chromatograms of ecgonine methyl ester samples. As listed in Tables 9-12, ecgonine methyl ester was not detected in any of the $T_{2\ years}$ samples tested. This may indicate two things. The first is that the method developed for this research was not able to isolate ecgonine methyl ester from the bone successfully. The second possibility is that there was no ecgonine methyl ester in the bones themselves after two years, meaning the sample was completely degraded. The second possibility seems plausible as the time zero sample has the largest concentration listed of all three compounds. The time zero bone proves that the method can successfully isolate ecgonine methyl ester from bone, but the compound is most likely not very stable in bone for longer periods of time.

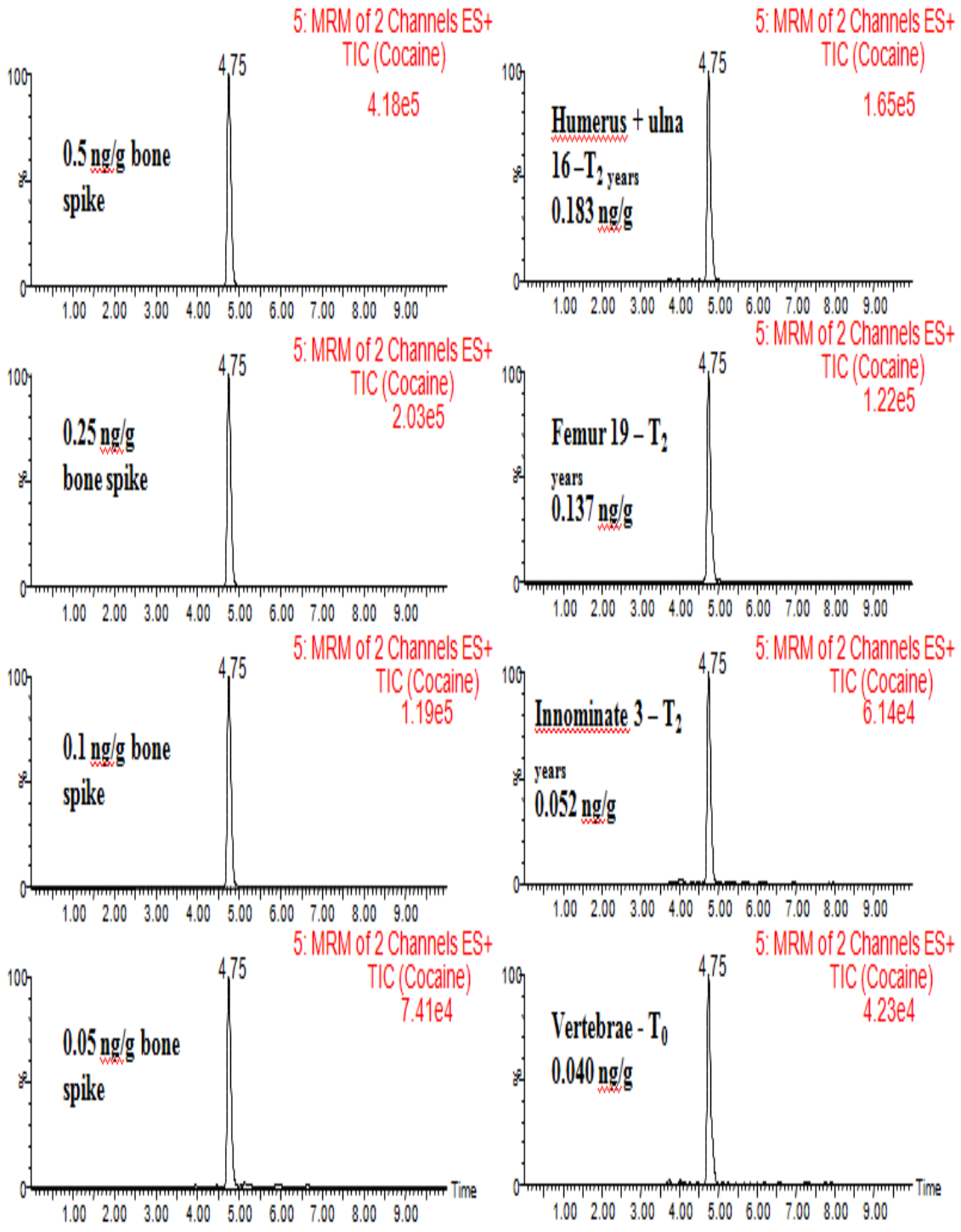



**Figure 16: Representative chromatograms of cocaine bone samples: spiked bone on left, unknown samples on right**

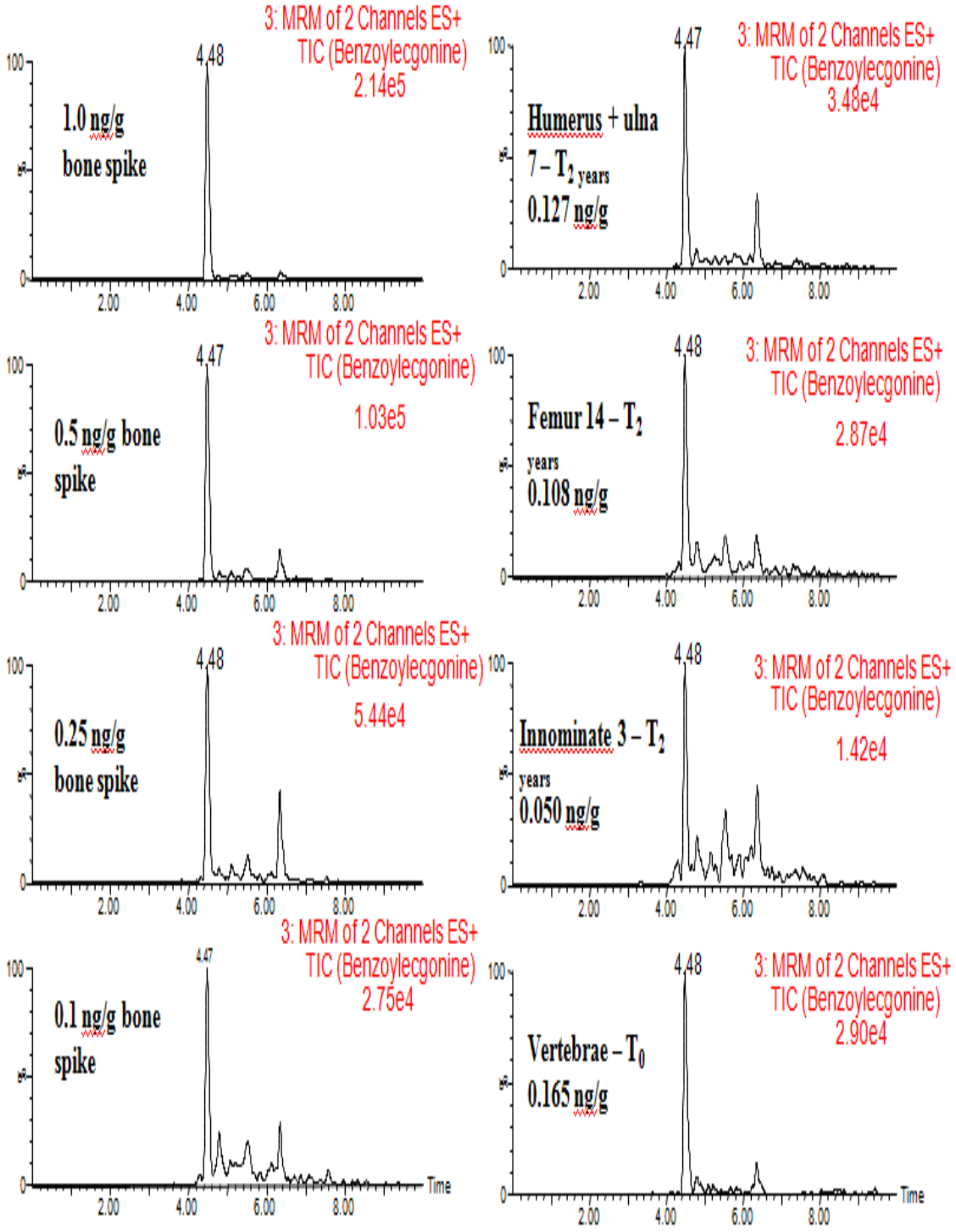



**Figure 17: Representative chromatograms of benzoylecgonine bone samples: spiked bone on left, unknown samples on right**

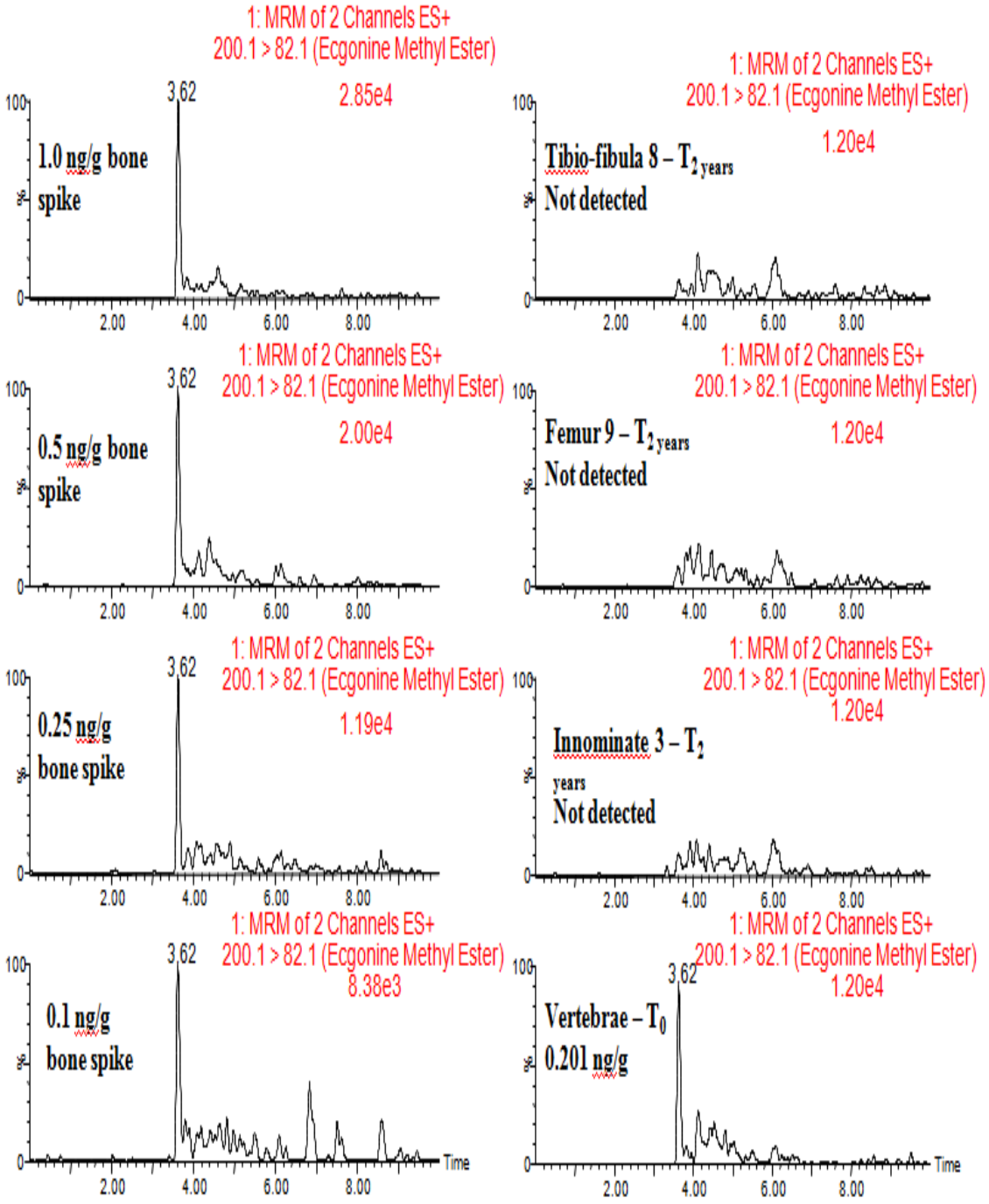



**Figure 18: Representative chromatograms of ecgonine methyl ester bone samples: spiked bone on left, unknown samples on right**

## 4. CONCLUSIONS

The product of this research was development of a rapid and robust method to accurately detect trace levels of cocaine in bone. In rodent bone samples that underwent decomposition for one year, followed by one year of storage, cocaine was detected at a range of 0-183pg/g or 0-0.183ng/g. Benzoylecgonine was detected at a range of 0-127pg/g or 0-0.127ng/g and ecgonine methyl ester was not detected in any of the two year samples. It was detected in a range of 50-201pg/g or 0.05-0.201ng/g in the time zero bone samples. Due to extraction efficiency issues for ecgonine methyl ester during the method development phase, it is unclear whether ecgonine methyl ester was totally absent from the bone samples or if the method was not strong enough to detect it. Cocaine has proven to remain detectable in bone after decomposition and storage at room temperature. Results that indicate trace level detection showcase a sharp, Gaussian peak shape for cocaine with a low baseline, however the concentration was well below the calibration curve generated for this data. With an even more sensitive mass spectrometer and a calibration curve encompassing lower concentrations, it would be possible to quantify cocaine in bone in the range of femtograms. This could be an advantage for forensic casework, since human skeletal remains are not often complete. So the ability to test any bone with confidence would be beneficial for future casework.

This methodology and instrumentation could be easily incorporated into toxicology testing laboratories. LC/MS is already an established and court approved method for forensic scientists. With the addition of a pump and a trap column, sample preparation times could be reduced tenfold and LOQ's and LOD's can be potentially

reduced. For forensic casework, which often has very time sensitive demands, this technology would be an asset to any laboratory.

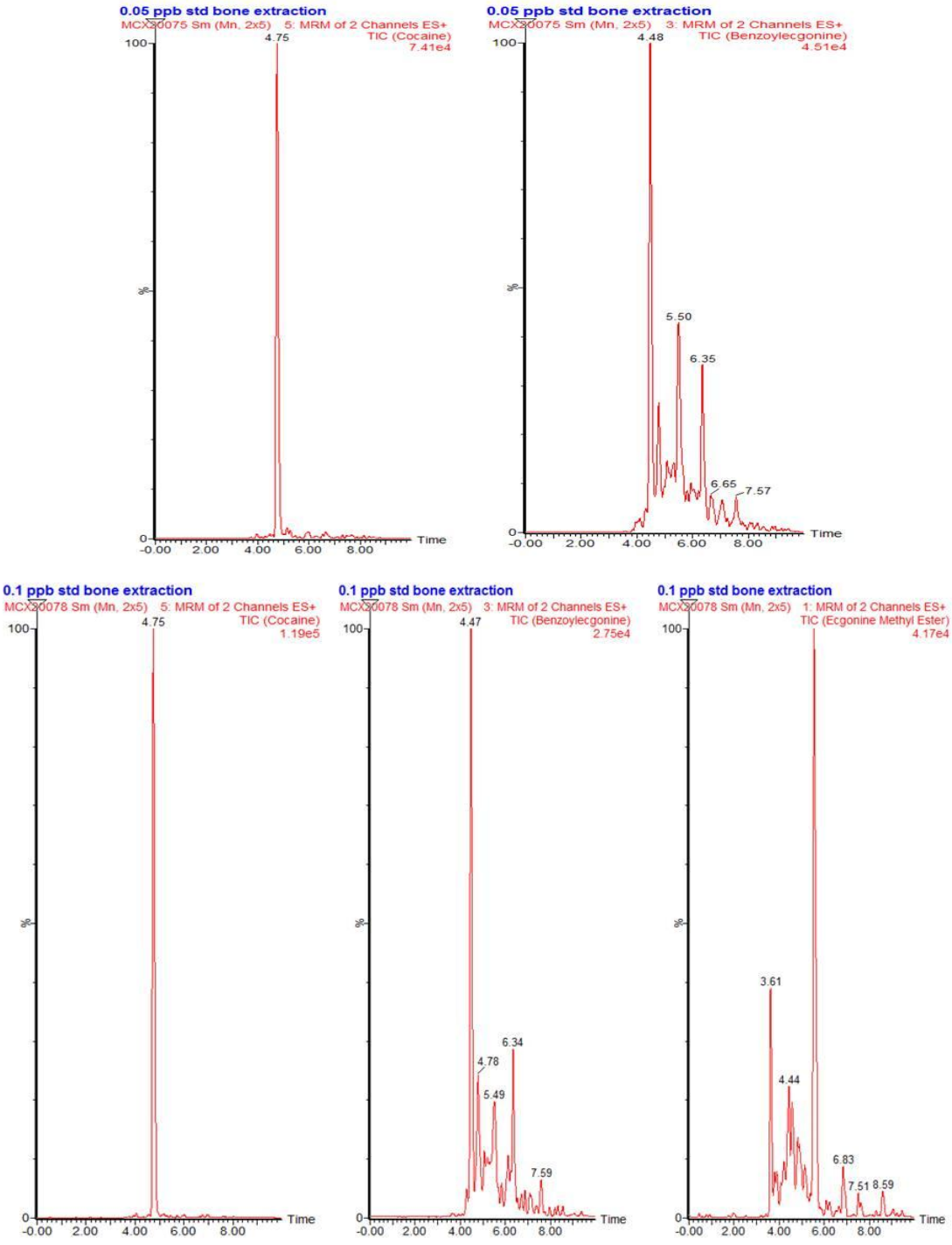
## 5. FUTURE DIRECTIONS

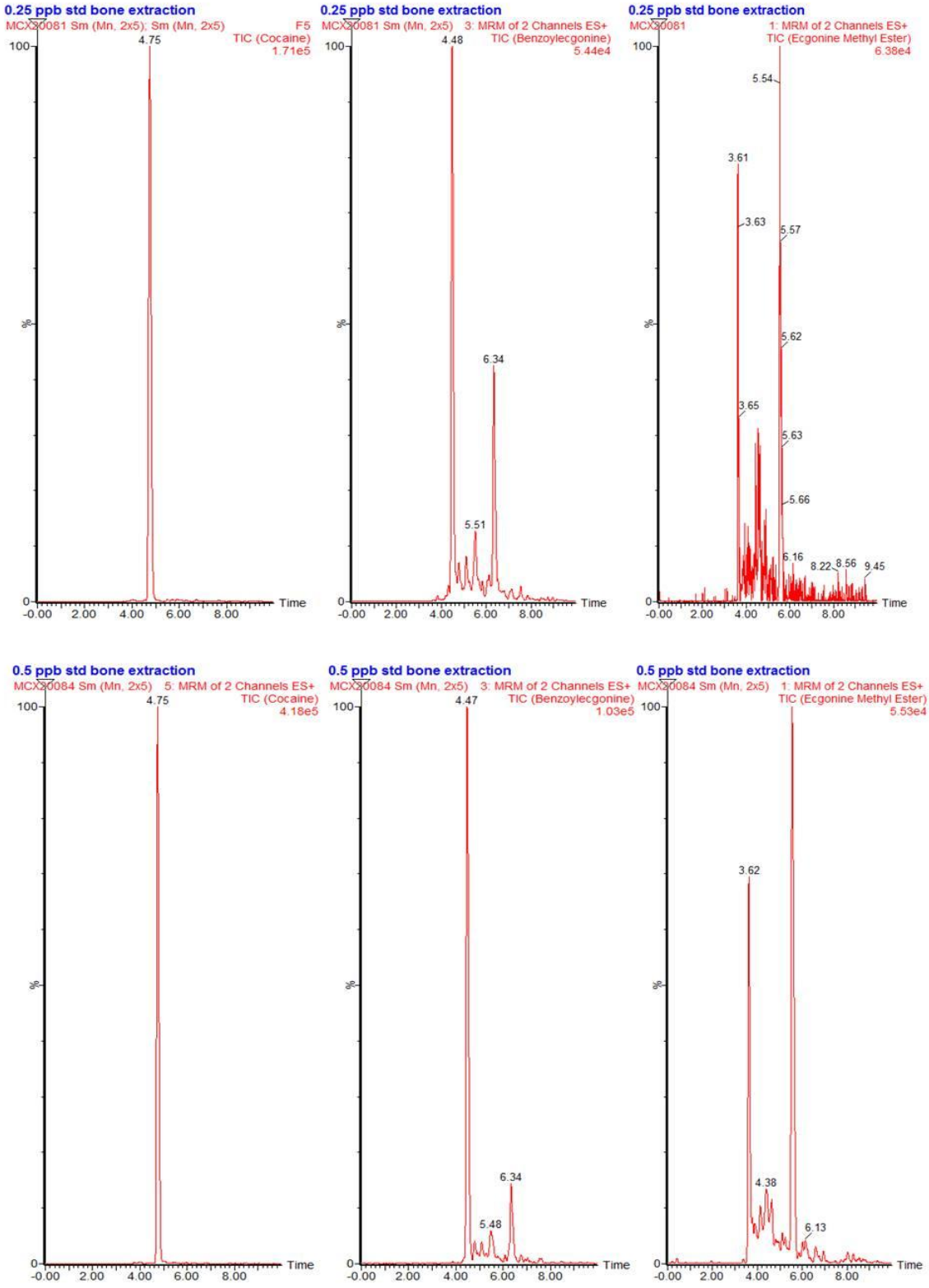
This project could be extended further in two main ways. The first option is to use the established method to test rodent bones that have decomposed for shorter periods of time. It would be interesting to see if shorter decomposition time leads to greater detectable concentrations in the bones. The second option is to optimize the method even further to achieve greater detection of the metabolites of cocaine. For example, the absence of ecgonine methyl ester in the bone samples does not necessarily indicate that there was no metabolite present. It is possible that the developed method was unable to successfully isolate the metabolite from the bone itself. This method could potentially improve by using a different solid phase extraction sorbent than the MCX cartridge. Ecgonine methyl ester and benzoylecgonine are both strong bases with pKa's around 10 or higher. It is possible that using an elution solvent at pH10 is not basic enough to neutralize these compounds enough to elute from the ion exchange portion of the sorbent. Instead, a weak ion exchange sorbent (WXC), could be used. The WXC sorbent contains carboxylic acid at a pKa ~ 5. For a method utilizing WCX, strong bases would be captured on the ion exchange portion, and the purpose of the elution solvent would be to neutralize the sorbent itself instead of the compounds. Therefore, all strong bases would elute, regardless of the compound's pKa.

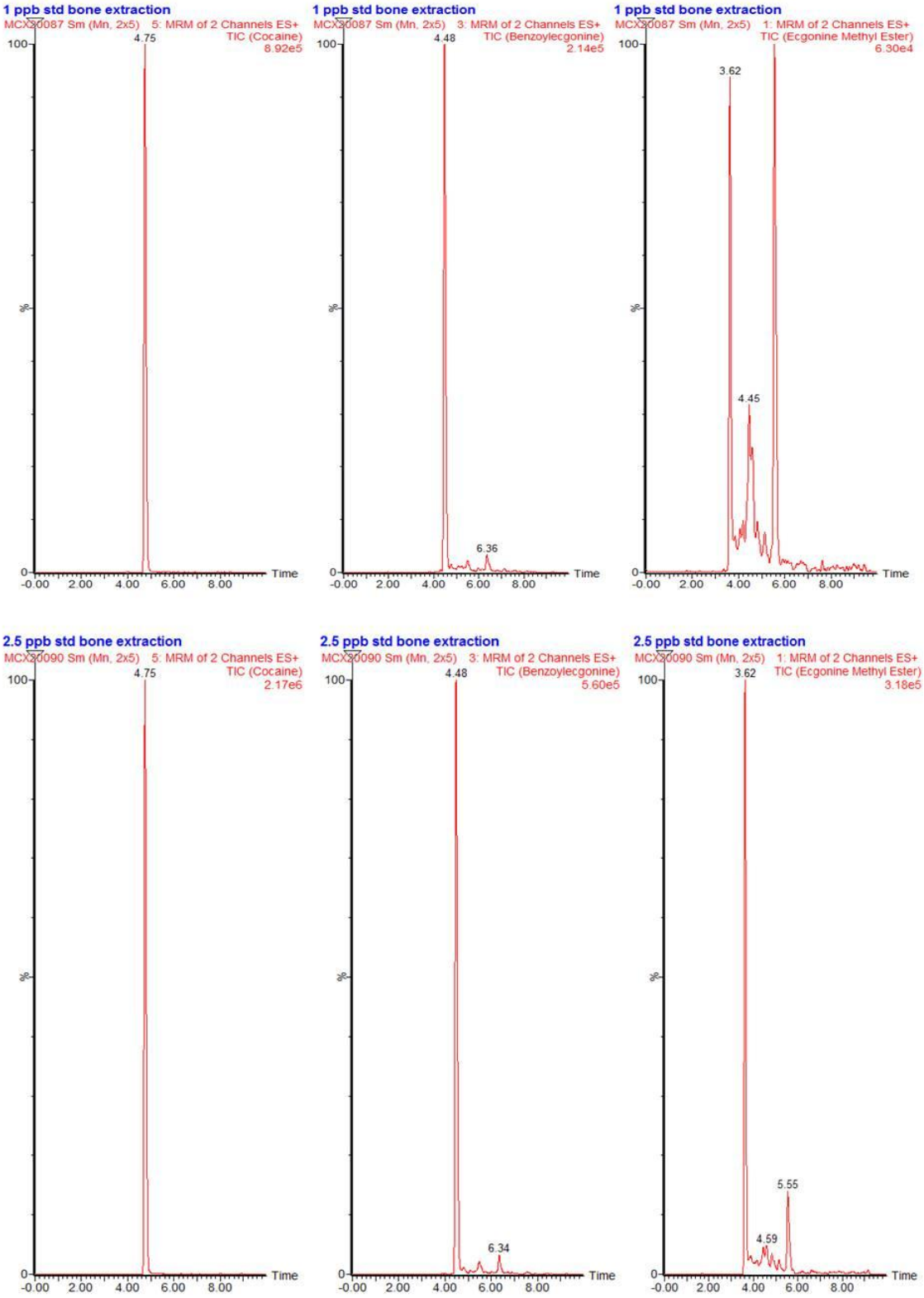
Another potential project would be to explore and compare detection of drugs in bone after one time usage and chronic usage of the drug of interest. Additionally, exploring other drugs in addition to cocaine in bone would be worthwhile for forensic investigations. Finally, from an anthropological point of view, it might be possible to

screen for substances anthropologists suspect may have been ingested on a daily bases in archaeological cases.

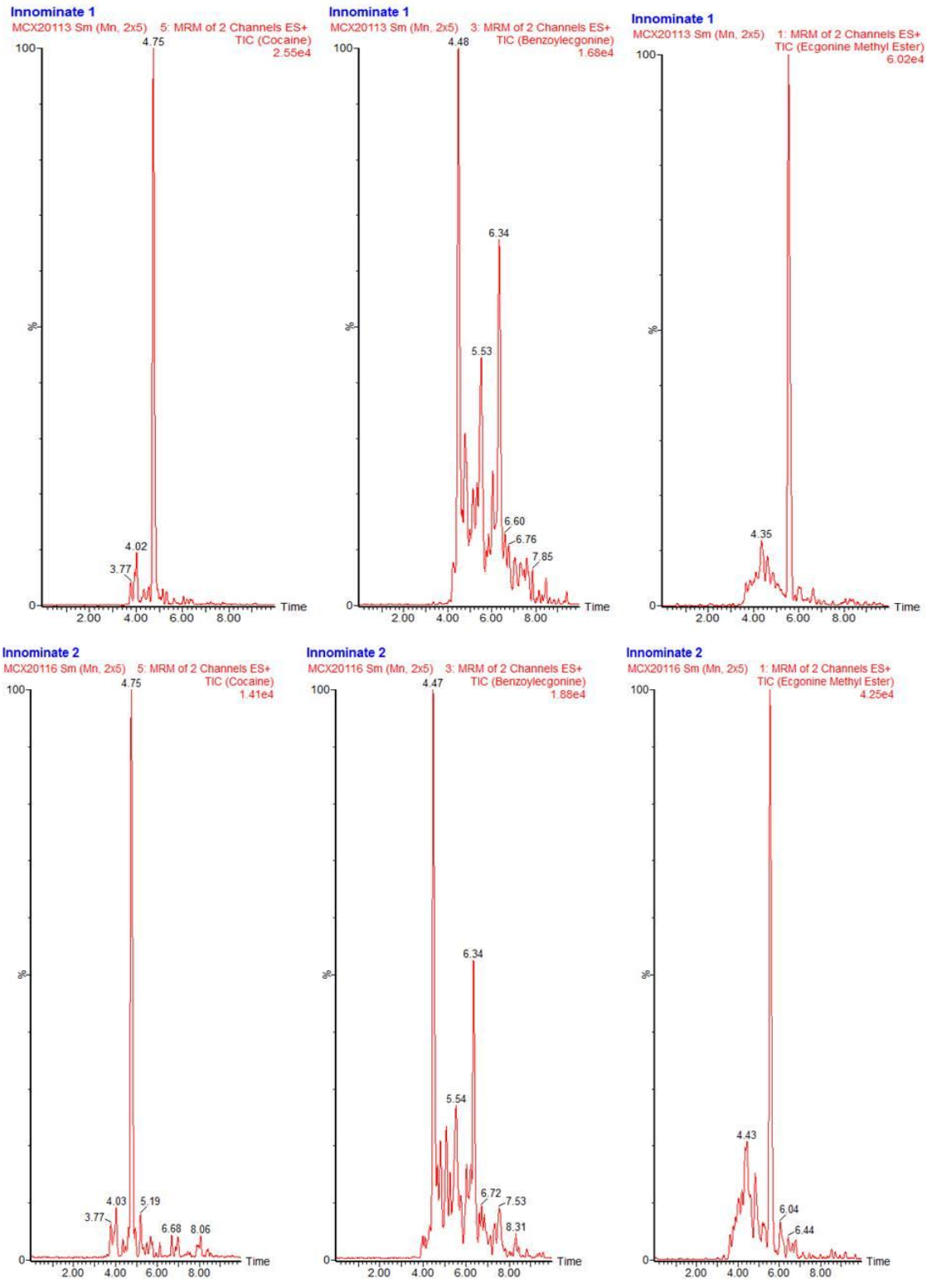
**APPENDIX A: Chromatograms of bone calibration curve and representative chromatograms of each sample bone type**

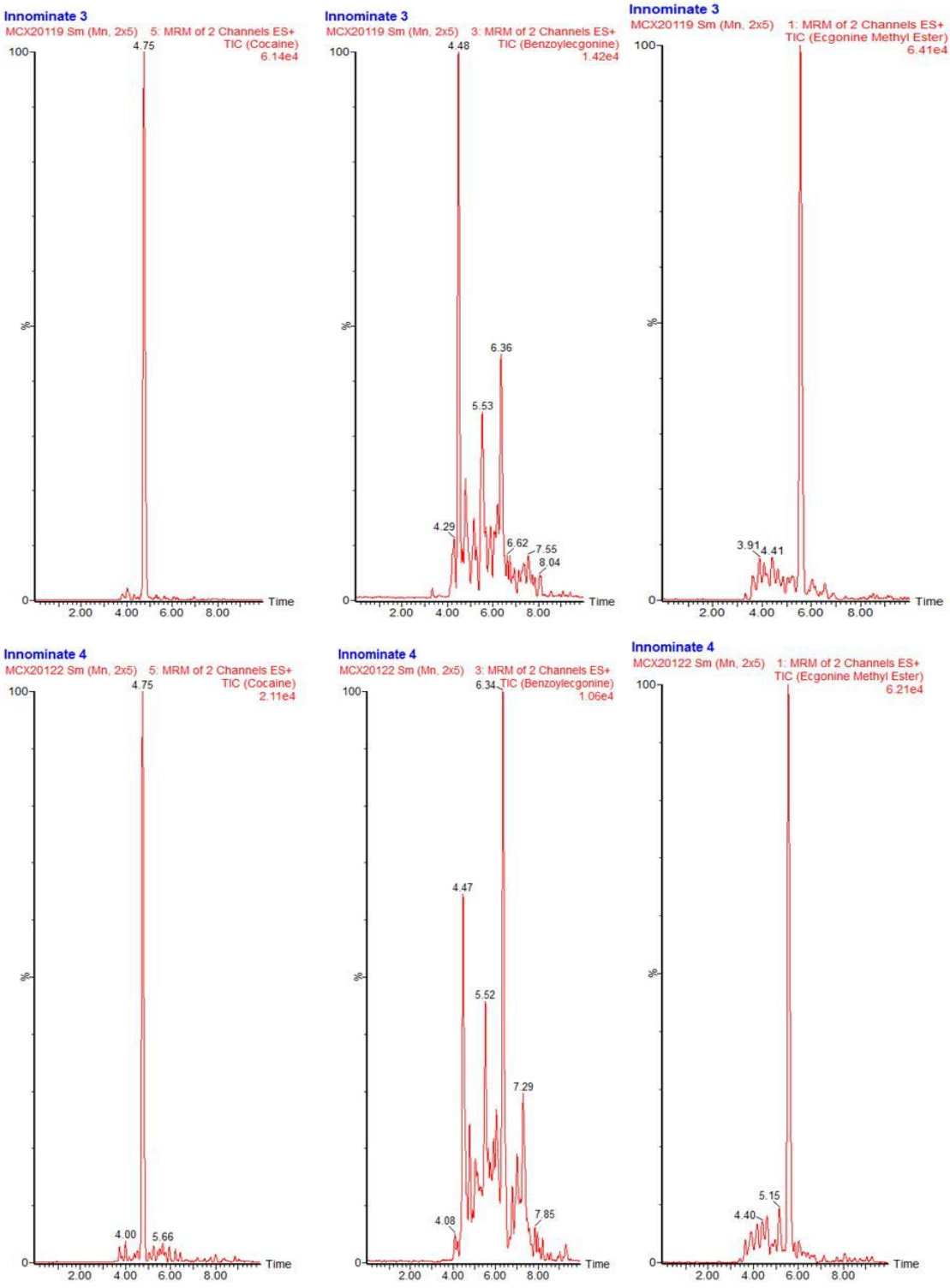


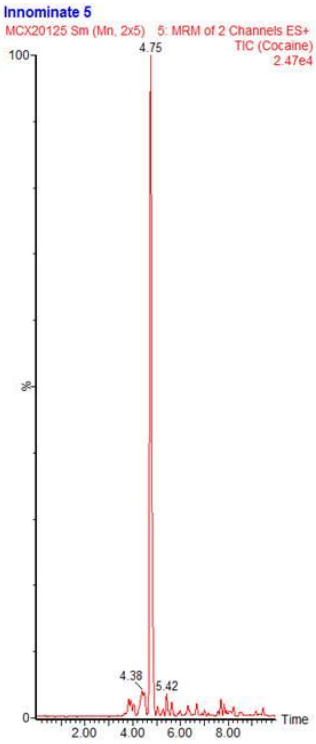

0.25 ppb std bone extraction
MCX30081 Sm (Mn, 2x5); Sm (Mn, 2x5) F5
TIC (Cocaine)
1.71e5
4.75
0.25 ppb std bone extraction
MCX30081 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
5.44e4
4.48
6.34
5.51
0.25 ppb std bone extraction
MCX30081 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.38e4
5.54
3.61
3.63
5.57
5.62
3.65
5.63
5.66
6.16
8.22
8.56
9.45
0.5 ppb std bone extraction
MCX30084 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
4.18e5
4.75
0.5 ppb std bone extraction
MCX30084 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.03e5
4.47
6.34
5.48
0.5 ppb std bone extraction
MCX30084 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.53e4
3.62
4.38
6.13
100
%
0
Time
-0.00
2.00
4.00
6.00
8.00

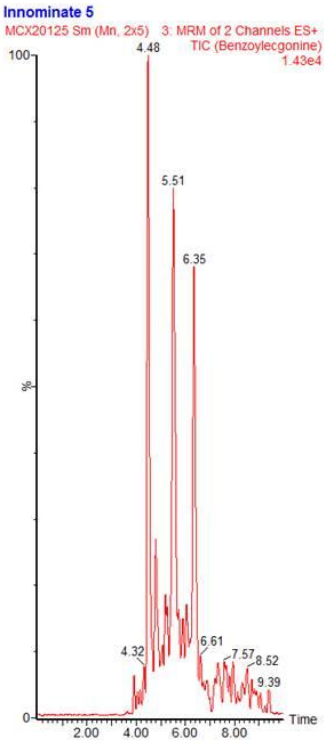

1 ppb std bone extraction
MCX20087 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
8.92e5
4.75
1 ppb std bone extraction
MCX20087 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
2.14e5
4.48
6.36
1 ppb std bone extraction
MCX20087 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.30e4
3.62
4.45
2.5 ppb std bone extraction
MCX20090 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
2.17e6
4.75
2.5 ppb std bone extraction
MCX20090 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
5.60e5
4.48
6.34
2.5 ppb std bone extraction
MCX20090 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
3.18e5
3.62
5.55
4.59
100
%
0
Time
-0.00 2.00 4.00 6.00 8.00

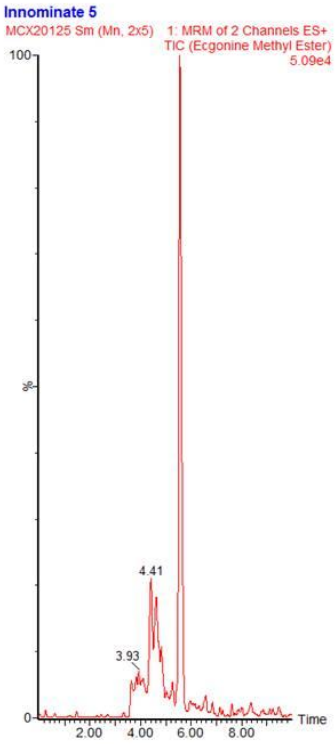

5 ppb std bone extraction
MCXS0093 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
4.21e6
4.75
5 ppb std bone extraction
MCXS0093 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.19e6
4.48
5 ppb std bone extraction
MCXS0093 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.53e5
3.61
5.55
10 ppb std bone extraction
MCXS0096 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
7.91e6
4.75
10 ppb std bone extraction
MCXS0096 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
2.00e6
4.47
10 ppb std bone extraction
MCXS0096 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
8.96e5
3.62
4.58
5.54
100
%
0
Time
-0.00
2.00
4.00
6.00
8.00

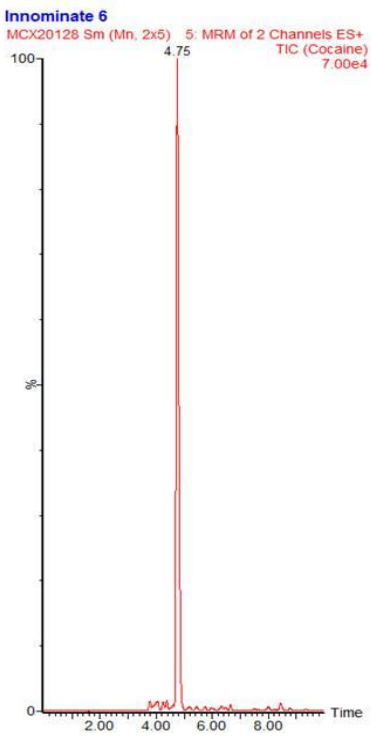

Innominate 1
MCX20113 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
2.55e4
4.75
4.02
3.77
Innominate 1
MCX20113 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.68e4
4.48
6.34
5.53
6.60
6.76
7.85
Innominate 1
MCX20113 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.02e4
4.35
Innominate 2
MCX20116 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
1.41e4
4.75
4.03
3.77
5.19
6.68
8.06
Innominate 2
MCX20116 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.88e4
4.47
6.34
5.54
6.72
7.53
8.31
Innominate 2
MCX20116 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
4.25e4
4.43
6.04
6.44
100
%
0
Time
2.00
4.00
6.00
8.00

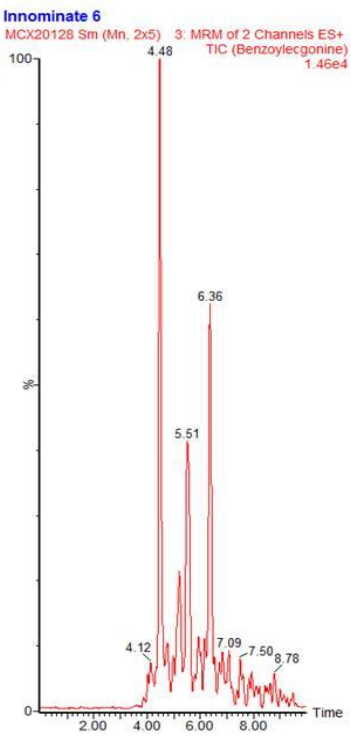

Innominate 3
MCX20119 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
6.14e4
4.75
Innominate 3
MCX20119 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.42e4
4.48
6.36
5.53
4.29
6.62
7.55
8.04
Innominate 3
MCX20119 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.41e4
3.91
4.41
Innominate 4
MCX20122 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
2.11e4
4.75
4.00
5.66
Innominate 4
MCX20122 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.06e4
6.34
4.47
5.52
7.29
4.08
7.85
Innominate 4
MCX20122 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.21e4
5.15
4.40
100
%
0
Time
2.00
4.00
6.00
8.00

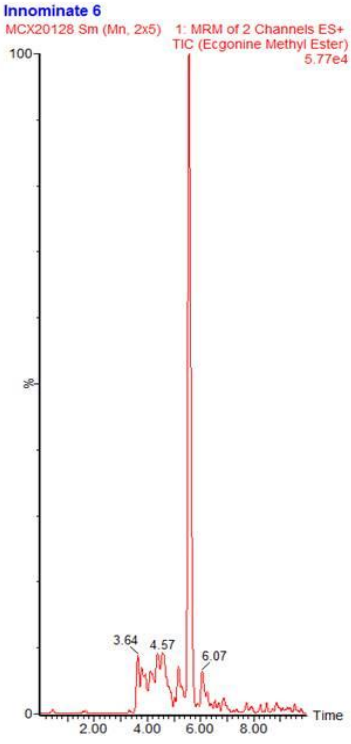

Innominate 5
MCX20125 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
2.47e4
4.75
4.38
5.42
100
%
0
2.00
4.00
6.00
8.00
Time

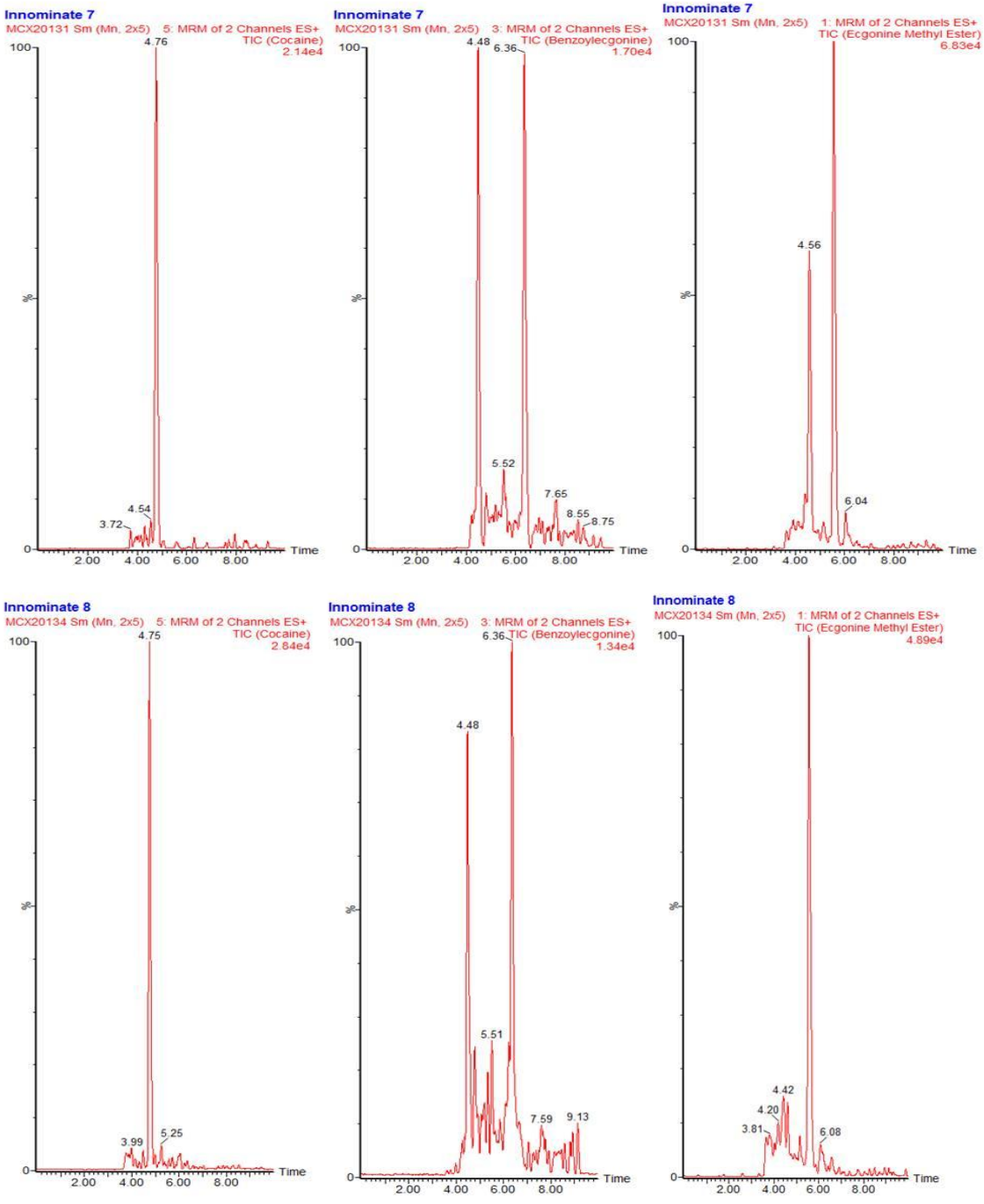

Innominate 5
MCX20125 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.43e4
4.48
5.51
6.35
4.32
6.61
7.57
8.52
9.39
100
%
0
2.00
4.00
6.00
8.00
Time

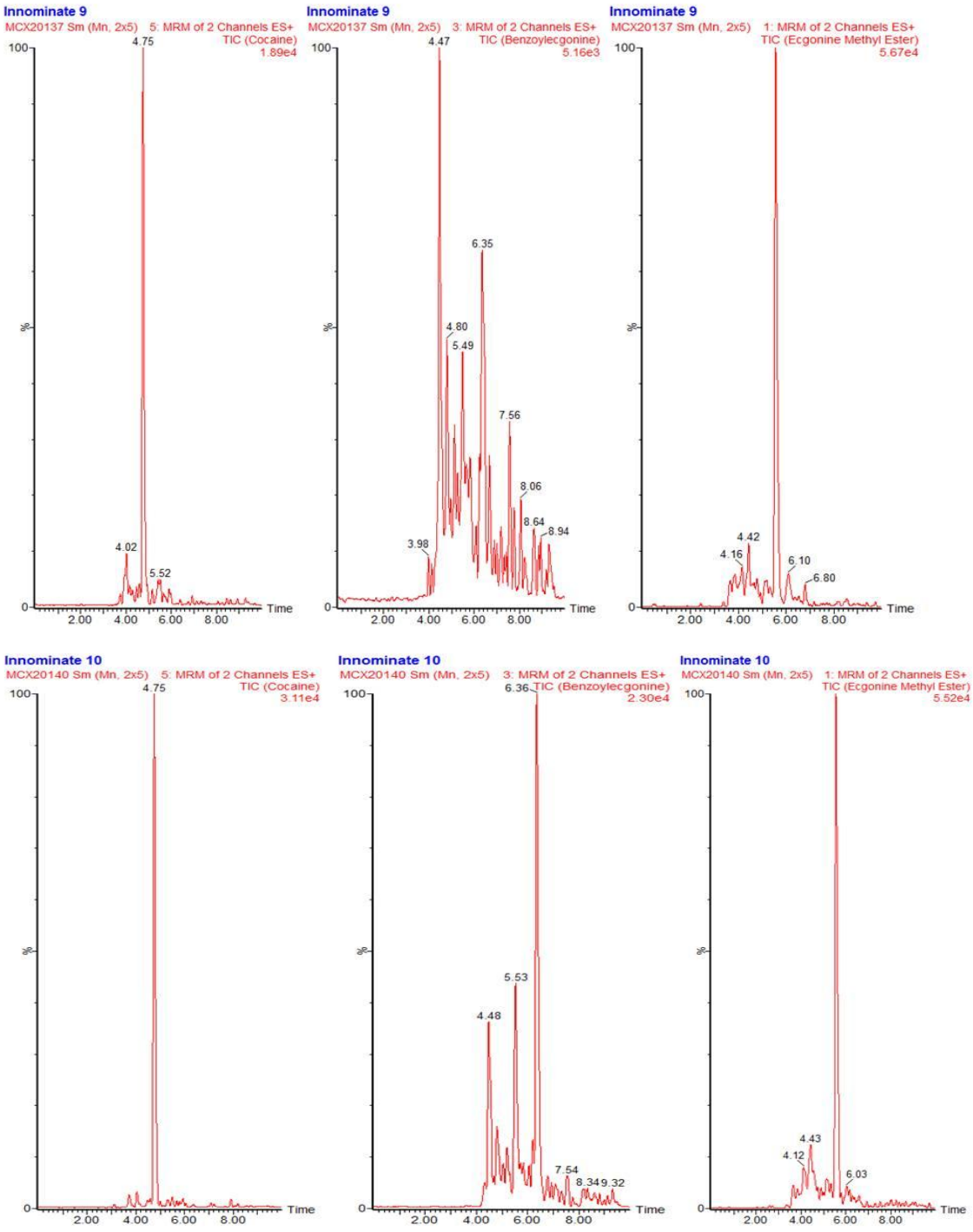

Innominate 5
MCX20125 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.09e4
4.41
3.93
100
%
0
2.00
4.00
6.00
8.00
Time

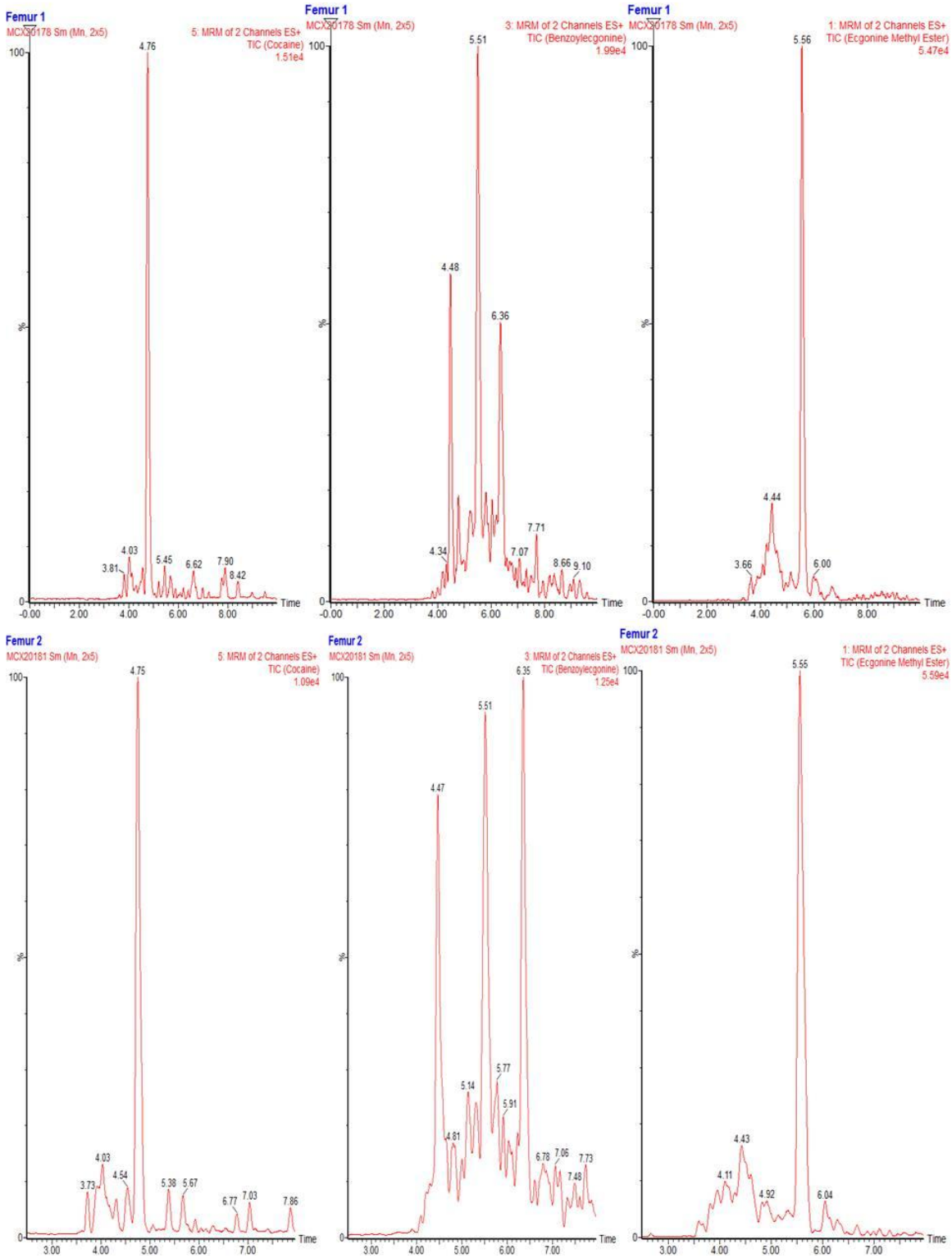

Innominate 6
MCX20128 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
7.00e4
4.75
100
%
0
2.00
4.00
6.00
8.00
Time

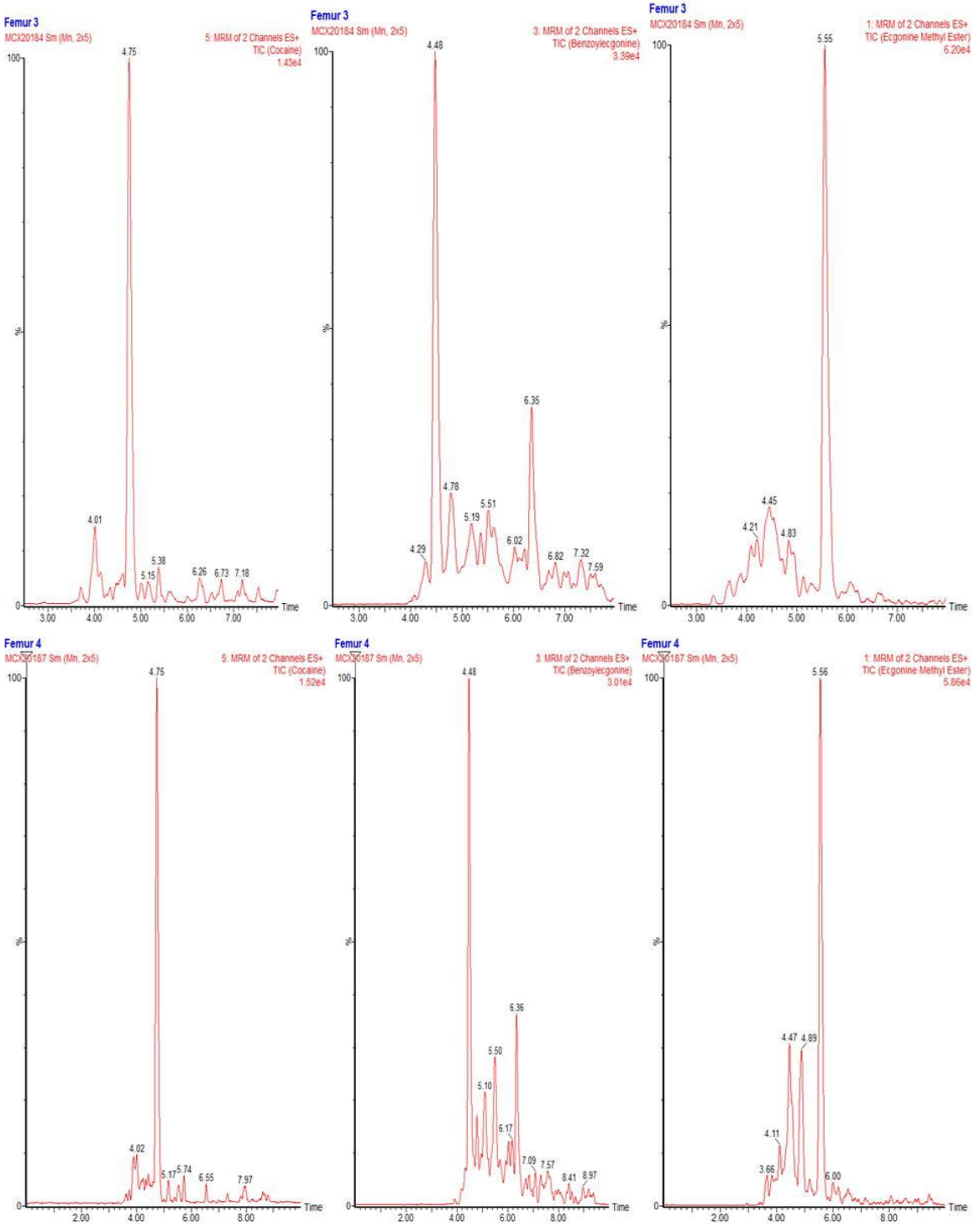

Innominate 6
MCX20128 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.46e4
4.48
6.36
5.51
4.12
7.09
7.50
8.78
100
%
0
2.00
4.00
6.00
8.00
Time

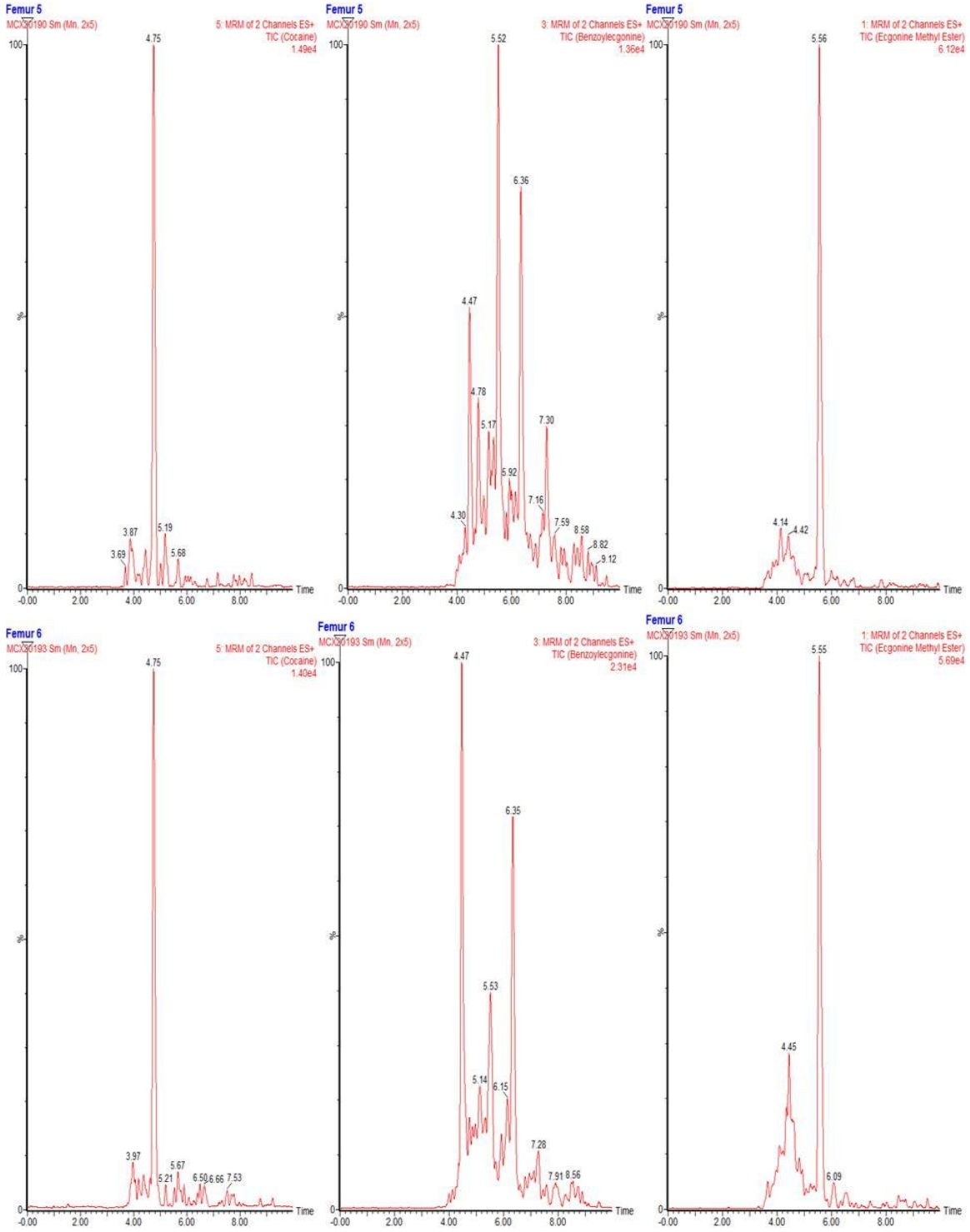

Innominate 6
MCX20128 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.77e4
3.64
4.57
6.07
100
%
0
2.00
4.00
6.00
8.00
Time

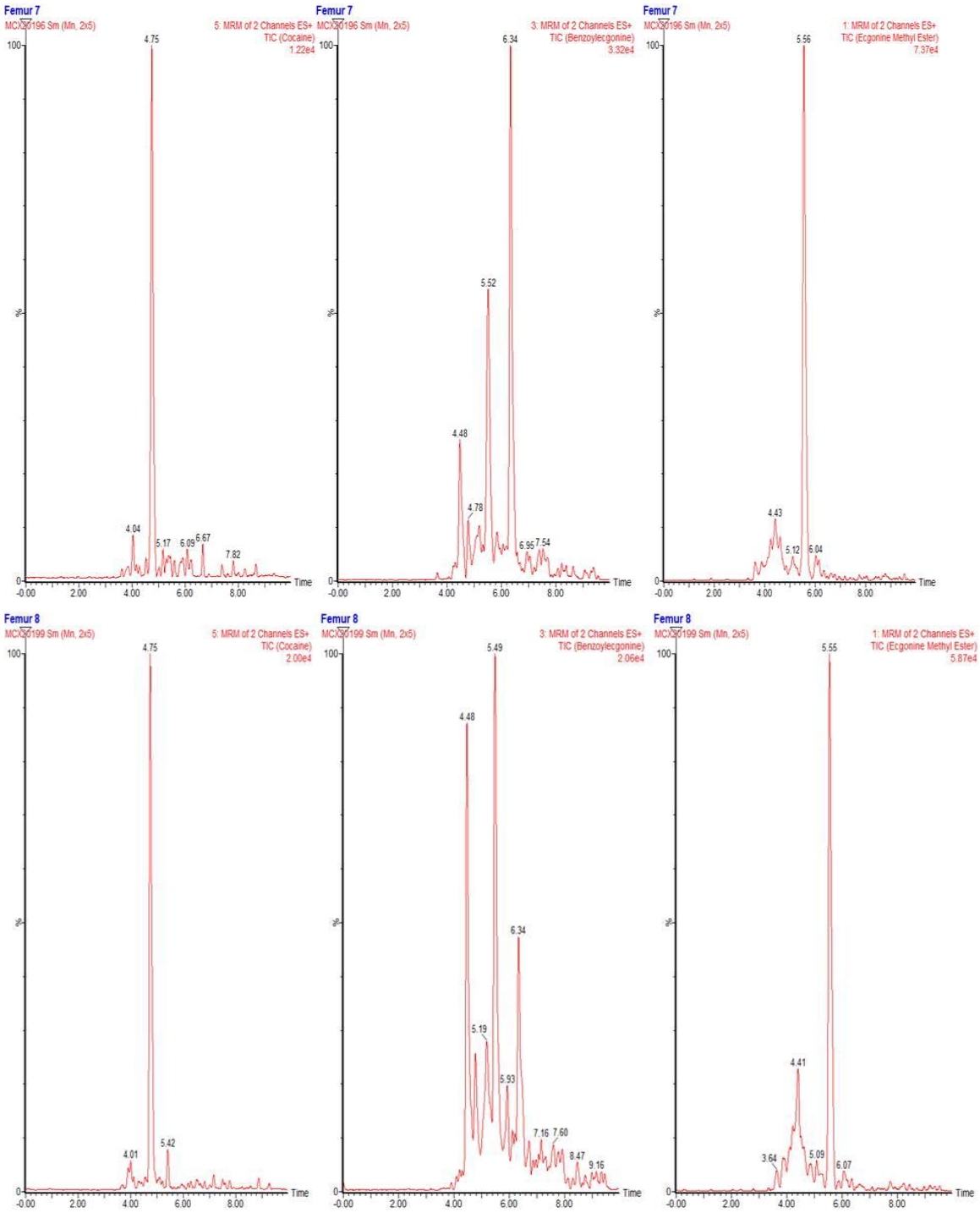

Innominate 7
MCX20131 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
2.14e4
4.76
4.54
3.72
Innominate 7
MCX20131 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.70e4
4.48
6.36
5.52
7.65
8.55
8.75
Innominate 7
MCX20131 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.83e4
4.56
6.04
Innominate 8
MCX20134 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+
TIC (Cocaine)
2.84e4
4.75
3.99
5.25
Innominate 8
MCX20134 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.34e4
6.36
4.48
5.51
7.59
9.13
Innominate 8
MCX20134 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
4.89e4
4.42
4.20
3.81
6.08
100
%
0
Time
2.00
4.00
6.00
8.00

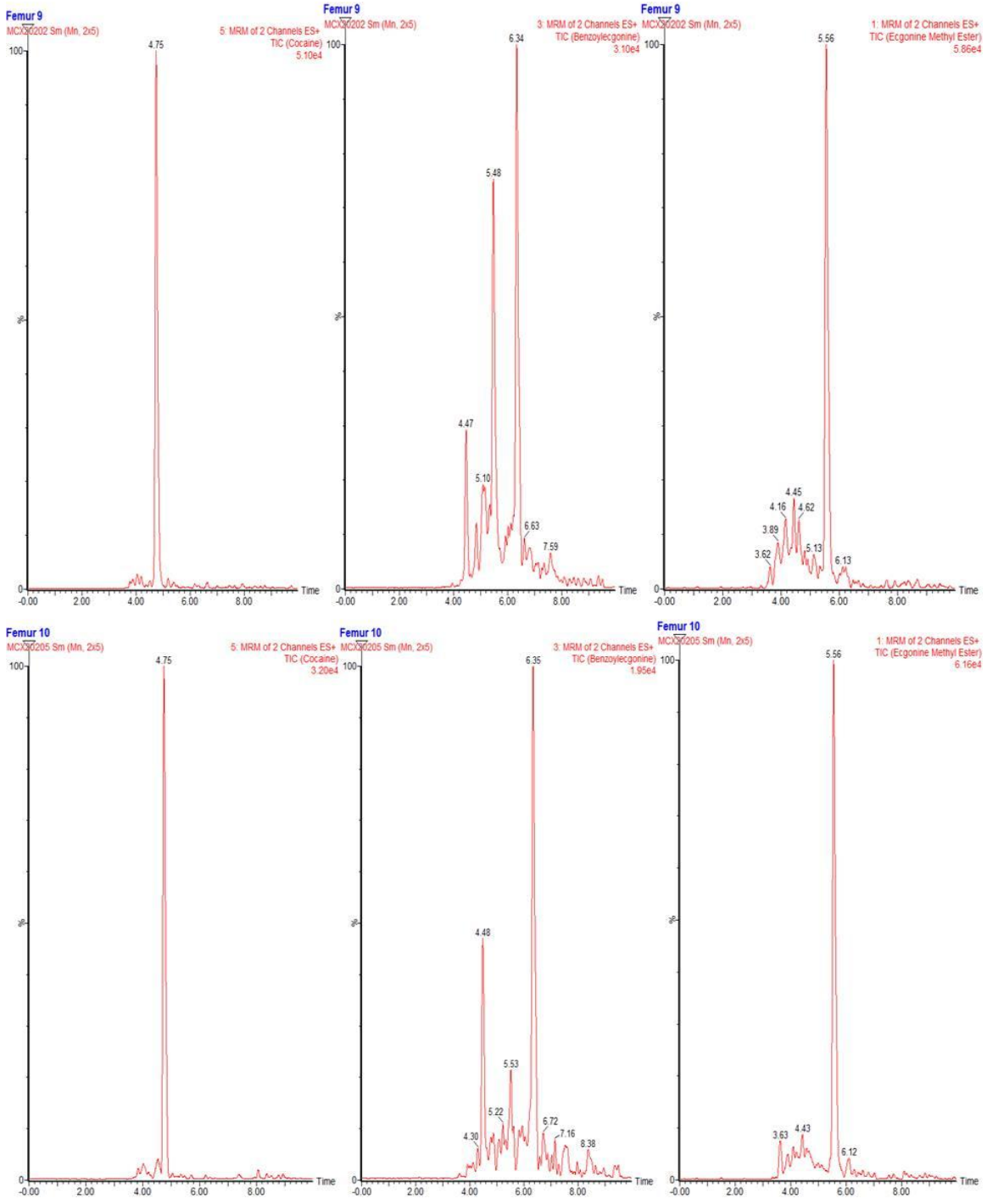

Innominate 9
MCX20137 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+ TIC (Cocaine) 1.89e4
4.75
4.02
5.52
Innominate 9
MCX20137 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+ TIC (Benzoylecgonine) 5.16e3
4.47
6.35
4.80
5.49
7.56
8.06
8.64
8.94
3.98
Innominate 9
MCX20137 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+ TIC (Ecgonine Methyl Ester) 5.67e4
4.42
4.16
6.10
6.80
Innominate 10
MCX20140 Sm (Mn, 2x5) 5: MRM of 2 Channels ES+ TIC (Cocaine) 3.11e4
4.75
Innominate 10
MCX20140 Sm (Mn, 2x5) 3: MRM of 2 Channels ES+ TIC (Benzoylecgonine) 2.30e4
6.36
5.53
4.48
7.54
8.34
9.32
Innominate 10
MCX20140 Sm (Mn, 2x5) 1: MRM of 2 Channels ES+ TIC (Ecgonine Methyl Ester) 5.52e4
4.43
4.12
6.03
100
%
0
Time
2.00
4.00
6.00
8.00

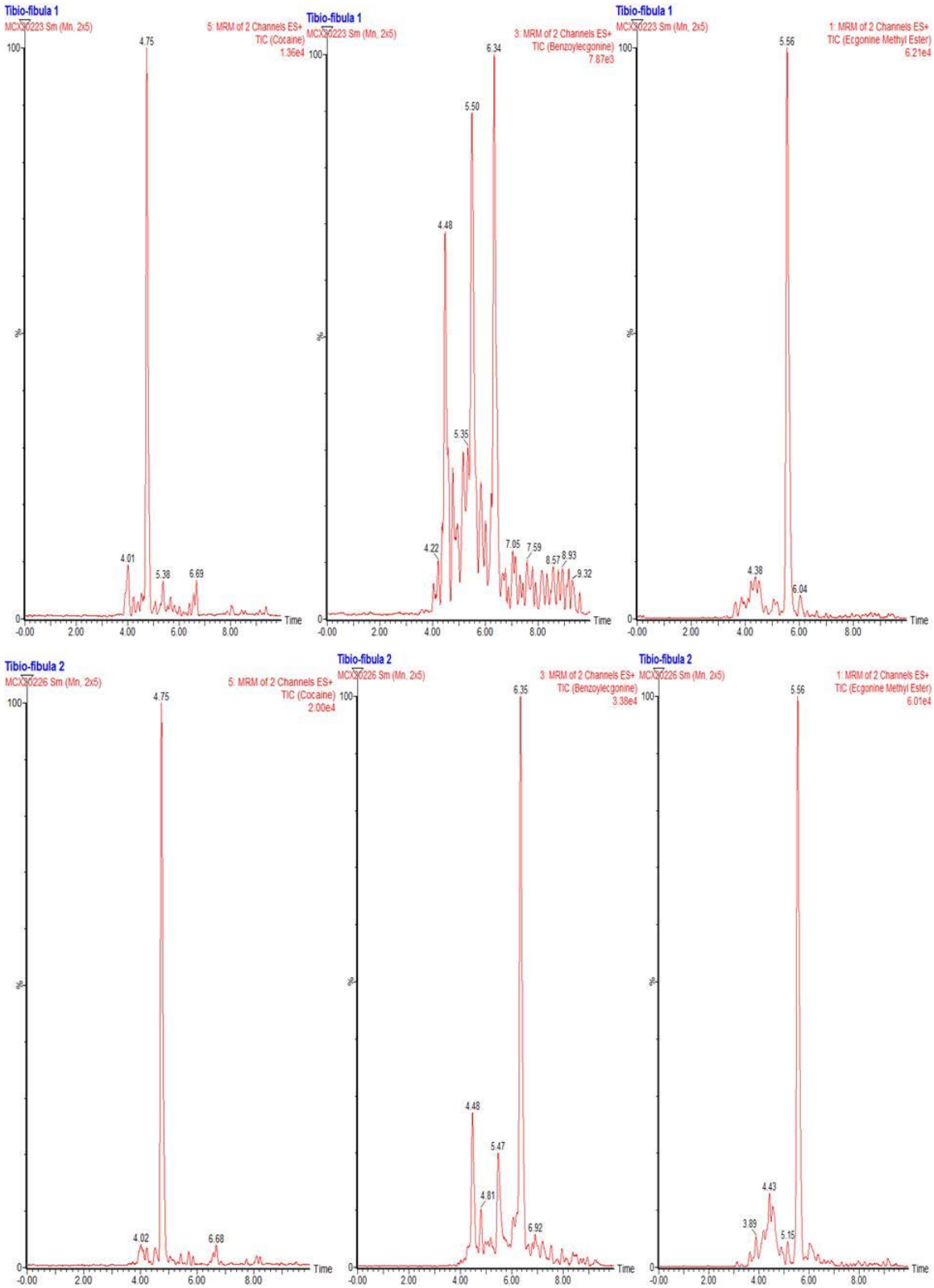

Femur 1
MCX20178 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.51e4
4.76
4.03
3.81
5.45
6.62
7.90
8.42
Femur 1
MCX20178 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.99e4
5.51
4.48
6.36
4.34
7.71
7.07
8.66
9.10
Femur 1
MCX20178 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.47e4
5.56
4.44
3.66
6.00
Femur 2
MCX20181 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.09e4
4.75
4.03
3.73
4.54
5.38
5.67
6.77
7.03
7.86
Femur 2
MCX20181 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.25e4
6.35
5.51
4.47
5.77
5.14
5.91
4.81
6.78
7.06
7.73
7.48
Femur 2
MCX20181 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.59e4
5.55
4.43
4.11
4.92
6.04
%
Time

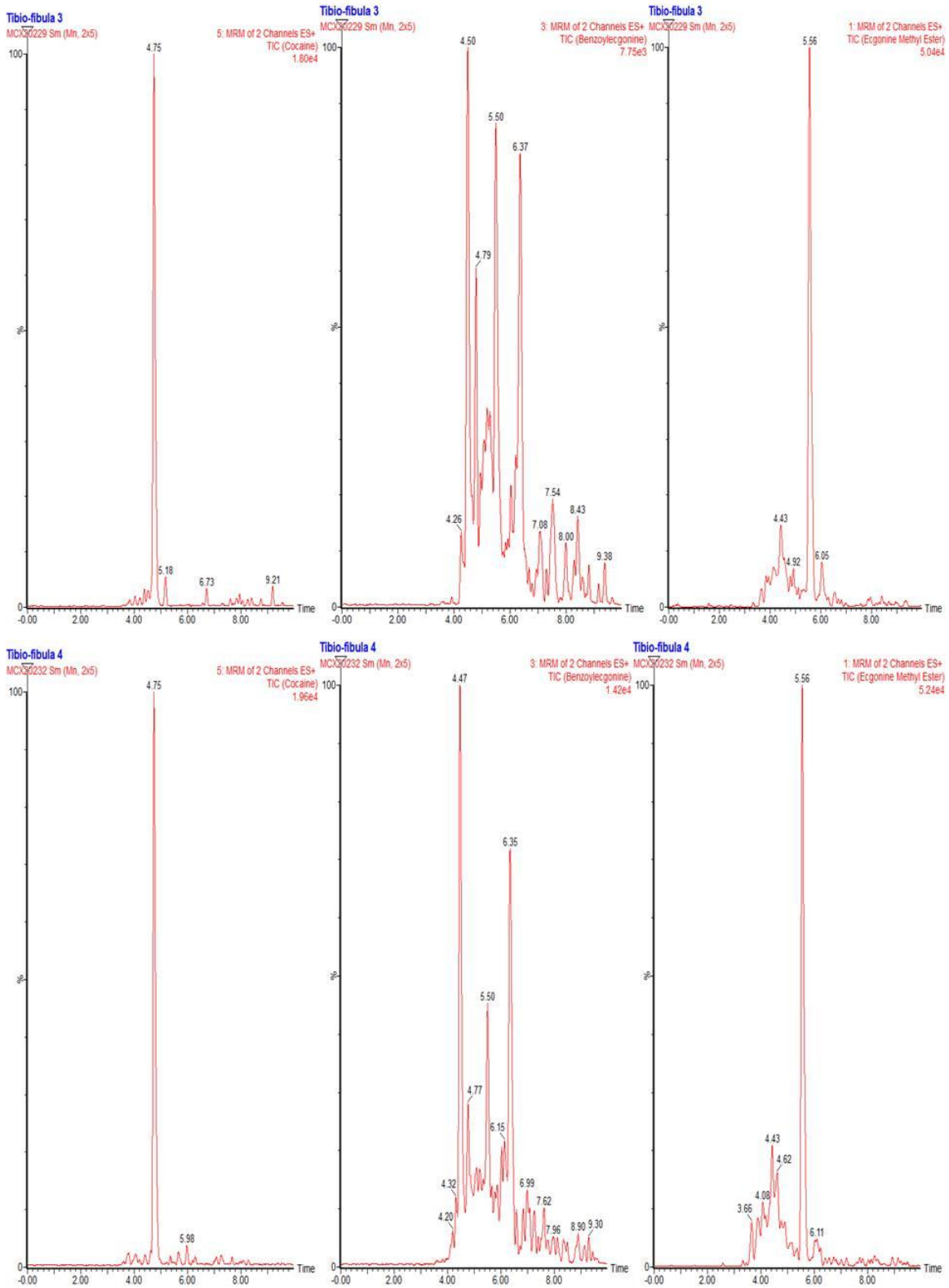

Femur 3
MCX20184 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.43e4
4.75
4.01
5.38
5.15
6.26
6.73
7.18
Femur 3
MCX20184 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
3.39e4
4.48
6.35
4.78
5.51
5.19
4.29
6.02
6.82
7.32
7.59
Femur 3
MCX20184 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.20e4
5.55
4.45
4.21
4.83
Femur 4
MCX20187 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.52e4
4.75
4.02
5.17
5.74
6.55
7.97
Femur 4
MCX20187 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
3.01e4
4.48
6.36
5.50
5.10
6.17
7.09
7.57
8.41
8.97
Femur 4
MCX20187 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.86e4
5.56
4.47
4.89
4.11
3.66
6.00
Time
%

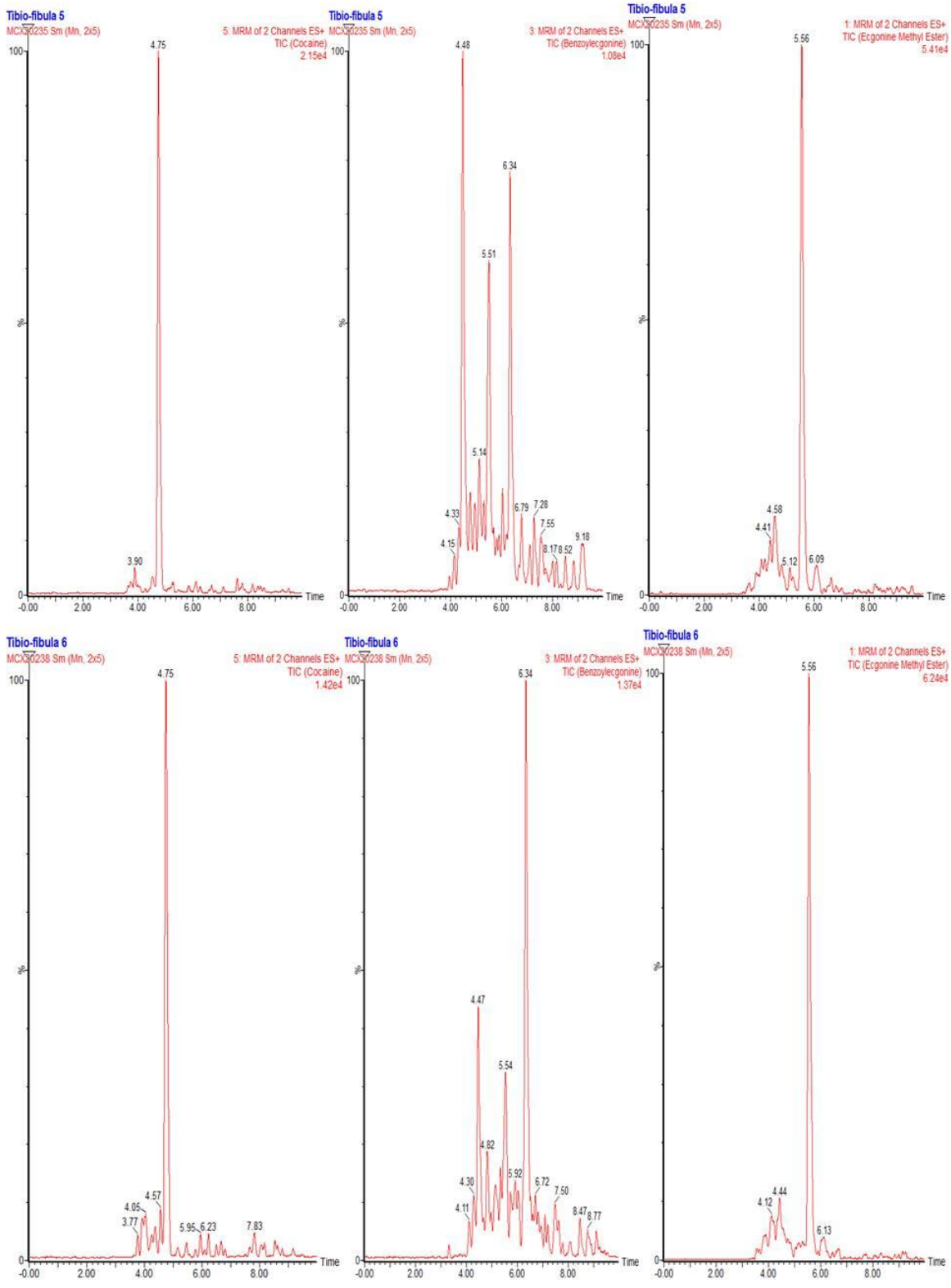

Femur 5
MCX0190 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.49e4
4.75
3.87
3.69
5.19
5.68
Femur 5
MCX0190 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.36e4
5.52
6.36
4.47
4.78
5.17
7.30
5.92
7.16
4.30
7.59
8.58
8.82
9.12
Femur 5
MCX0190 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.12e4
5.56
4.14
4.42
Femur 6
MCX0193 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.40e4
4.75
3.97
5.21
5.67
6.50
6.66
7.53
Femur 6
MCX0193 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
2.31e4
4.47
6.35
5.53
5.14
6.15
7.28
7.91
8.56
Femur 6
MCX0193 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.69e4
5.55
4.45
6.09
%
Time

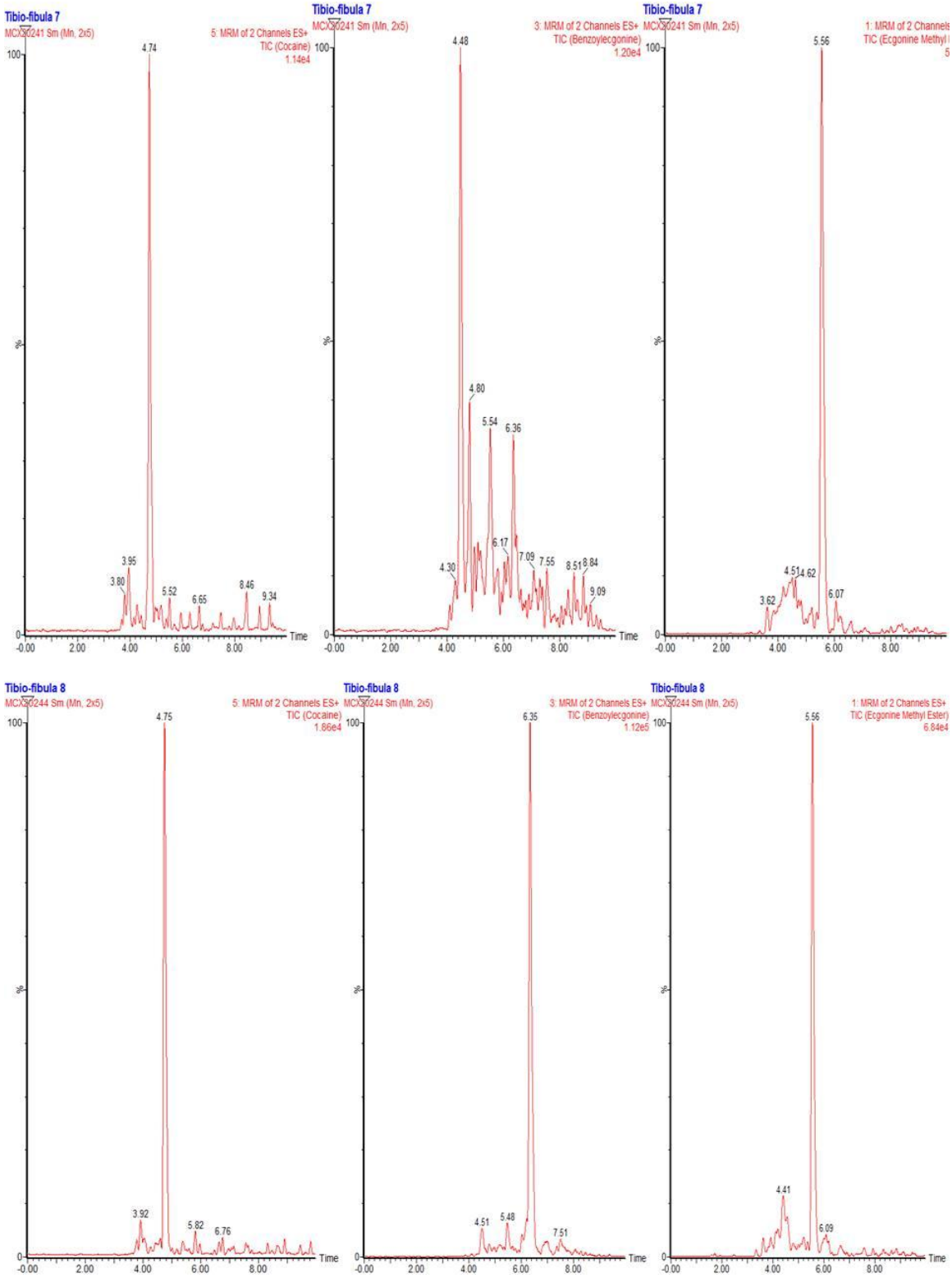

Femur 7
MCX20196 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.22e4
4.75
4.04
5.17
6.09
6.67
7.82
Femur 7
MCX20196 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
3.32e4
6.34
5.52
4.48
4.78
6.95
7.54
Femur 7
MCX20196 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
7.37e4
5.56
4.43
5.12
6.04
Femur 8
MCX20199 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
2.00e4
4.75
4.01
5.42
Femur 8
MCX20199 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
2.06e4
5.49
4.48
6.34
5.19
5.93
7.16
7.60
8.47
9.16
Femur 8
MCX20199 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.87e4
5.55
4.41
3.64
5.09
6.07
Time
%

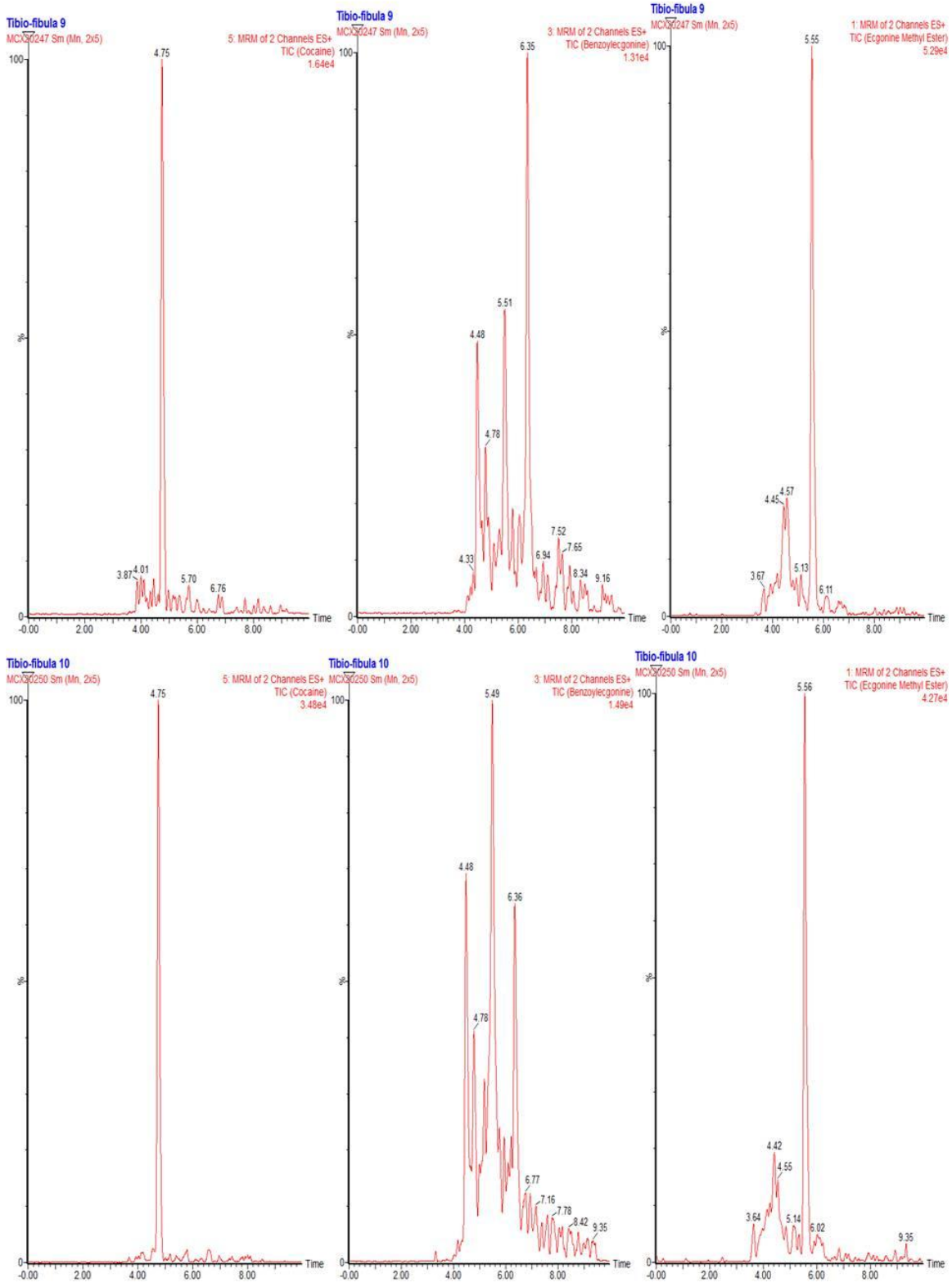

Femur 9
MCX0202 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
5.10e4
4.75
Femur 9
MCX0202 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
3.10e4
6.34
5.48
4.47
5.10
6.63
7.59
Femur 9
MCX0202 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.86e4
5.56
4.45
4.16
4.62
3.89
3.62
5.13
6.13
Femur 10
MCX0205 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
3.20e4
4.75
Femur 10
MCX0205 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.95e4
6.35
4.48
5.53
5.22
6.72
7.16
4.30
8.38
Femur 10
MCX0205 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.16e4
5.56
3.63
4.43
6.12
Time
%


Tibio-fibula 1
MCX0223 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.36e4
4.75
4.01
5.38
6.69
Tibio-fibula 1
MCX0223 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
7.87e3
6.34
5.50
4.48
5.35
4.22
7.05
7.59
8.57
8.93
9.32
Tibio-fibula 1
MCX0223 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.21e4
5.56
4.38
6.04
Tibio-fibula 2
MCX0226 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
2.00e4
4.75
4.02
6.68
Tibio-fibula 2
MCX0226 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
3.38e4
6.35
4.48
5.47
4.81
6.92
Tibio-fibula 2
MCX0226 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.01e4
5.56
4.43
3.89
5.15
%
Time


Tibio-fibula 3
MCX20229 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.80e4
4.75
5.18
6.73
9.21
Tibio-fibula 3
MCX20229 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
7.75e3
4.50
5.50
6.37
4.79
4.26
7.54
8.43
7.08
8.00
9.38
Tibio-fibula 3
MCX20229 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.04e4
5.56
4.43
4.92
6.05
Tibio-fibula 4
MCX20232 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.96e4
4.75
5.98
Tibio-fibula 4
MCX20232 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.42e4
4.47
6.35
5.50
4.77
6.15
4.32
4.20
6.99
7.62
7.96
8.90
9.30
Tibio-fibula 4
MCX20232 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.24e4
5.56
4.43
4.62
4.08
3.66
6.11
%
Time
0
100
-0.00
2.00
4.00
6.00
8.00


Tibio-fibula 5
MCX30235 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
2.15e4
4.75
3.90
Tibio-fibula 5
MCX30235 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.08e4
4.48
6.34
5.51
5.14
4.33
4.15
6.79
7.28
7.55
8.17
8.52
9.18
Tibio-fibula 5
MCX30235 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.41e4
5.56
4.58
4.41
5.12
6.09
Tibio-fibula 6
MCX30238 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.42e4
4.75
4.57
4.05
3.77
5.95
6.23
7.83
Tibio-fibula 6
MCX30238 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.37e4
6.34
4.47
5.54
4.82
5.92
4.30
4.11
6.72
7.50
8.47
8.77
Tibio-fibula 6
MCX30238 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.24e4
5.56
4.44
4.12
6.13
%
Time
0
100
-0.00
2.00
4.00
6.00
8.00


Tibio-fibula 7
MCX20241 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.14e4
4.74
3.95
3.80
5.52
6.65
8.46
9.34
Tibio-fibula 7
MCX20241 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.20e4
4.48
4.80
5.54
6.36
6.17
4.30
7.09
7.55
8.51
8.84
9.09
Tibio-fibula 7
MCX20241 Sm (Mn, 2x5)
1: MRM of 2 Channels
TIC (Ecgonine Methyl
5.56
4.51
4.62
3.62
6.07
Tibio-fibula 8
MCX20244 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.86e4
4.75
3.92
5.82
6.76
Tibio-fibula 8
MCX20244 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.12e5
6.35
4.51
5.48
7.51
Tibio-fibula 8
MCX20244 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.84e4
5.56
4.41
6.09
Time
%


Tibio-fibula 9
MCX0247 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.64e4
4.75
3.87
4.01
5.70
6.76
Tibio-fibula 9
MCX0247 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.31e4
6.35
5.51
4.48
4.78
4.33
6.94
7.52
7.65
8.34
9.16
Tibio-fibula 9
MCX0247 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.29e4
5.55
4.45
4.57
3.67
5.13
6.11
Tibio-fibula 10
MCX0250 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
3.48e4
4.75
Tibio-fibula 10
MCX0250 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.49e4
5.49
4.48
6.36
4.78
6.77
7.16
7.78
8.42
9.35
Tibio-fibula 10
MCX0250 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
4.27e4
5.56
4.42
4.55
3.64
5.14
6.02
9.35
%
Time
-0.00
2.00
4.00
6.00
8.00
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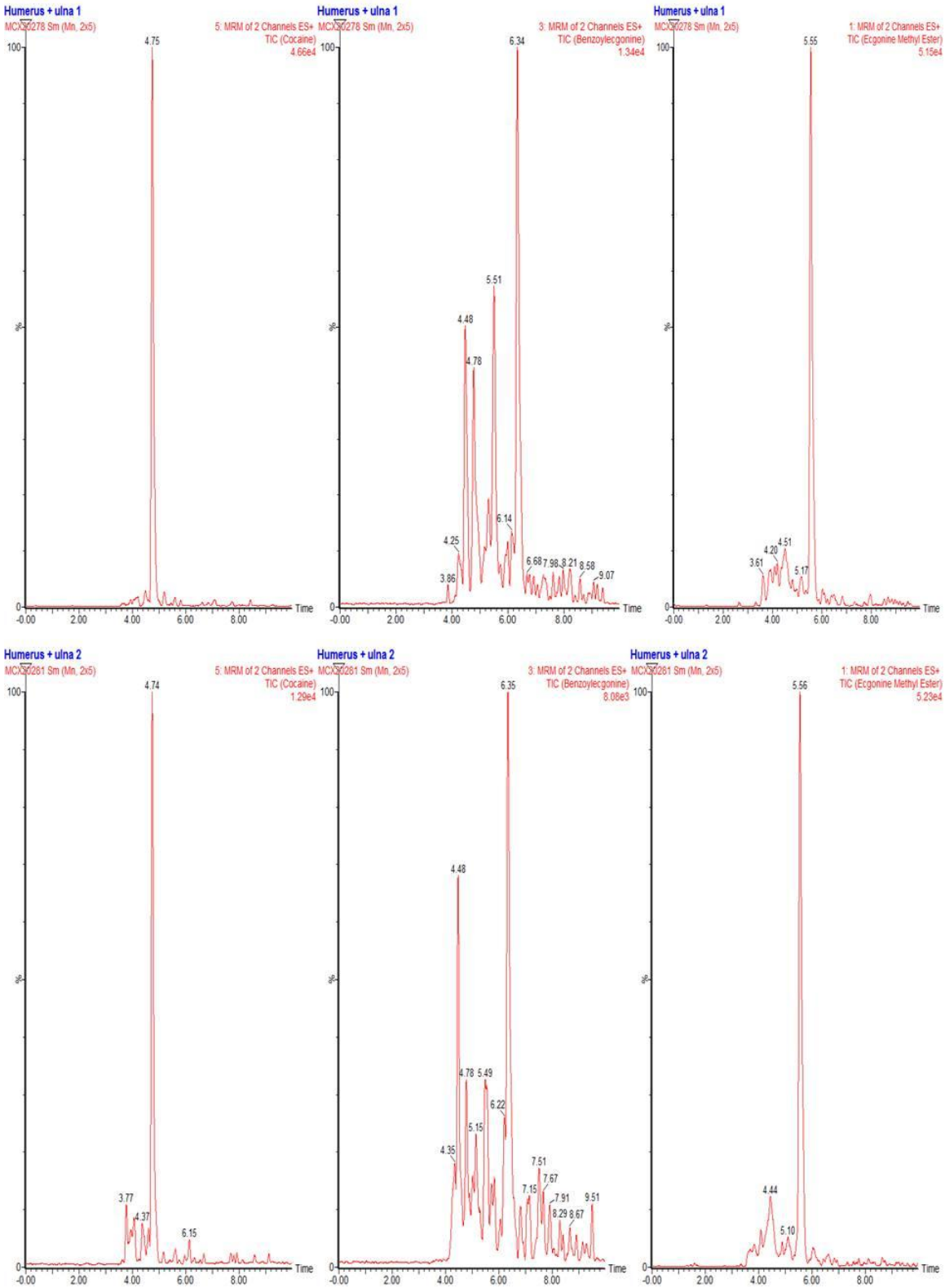
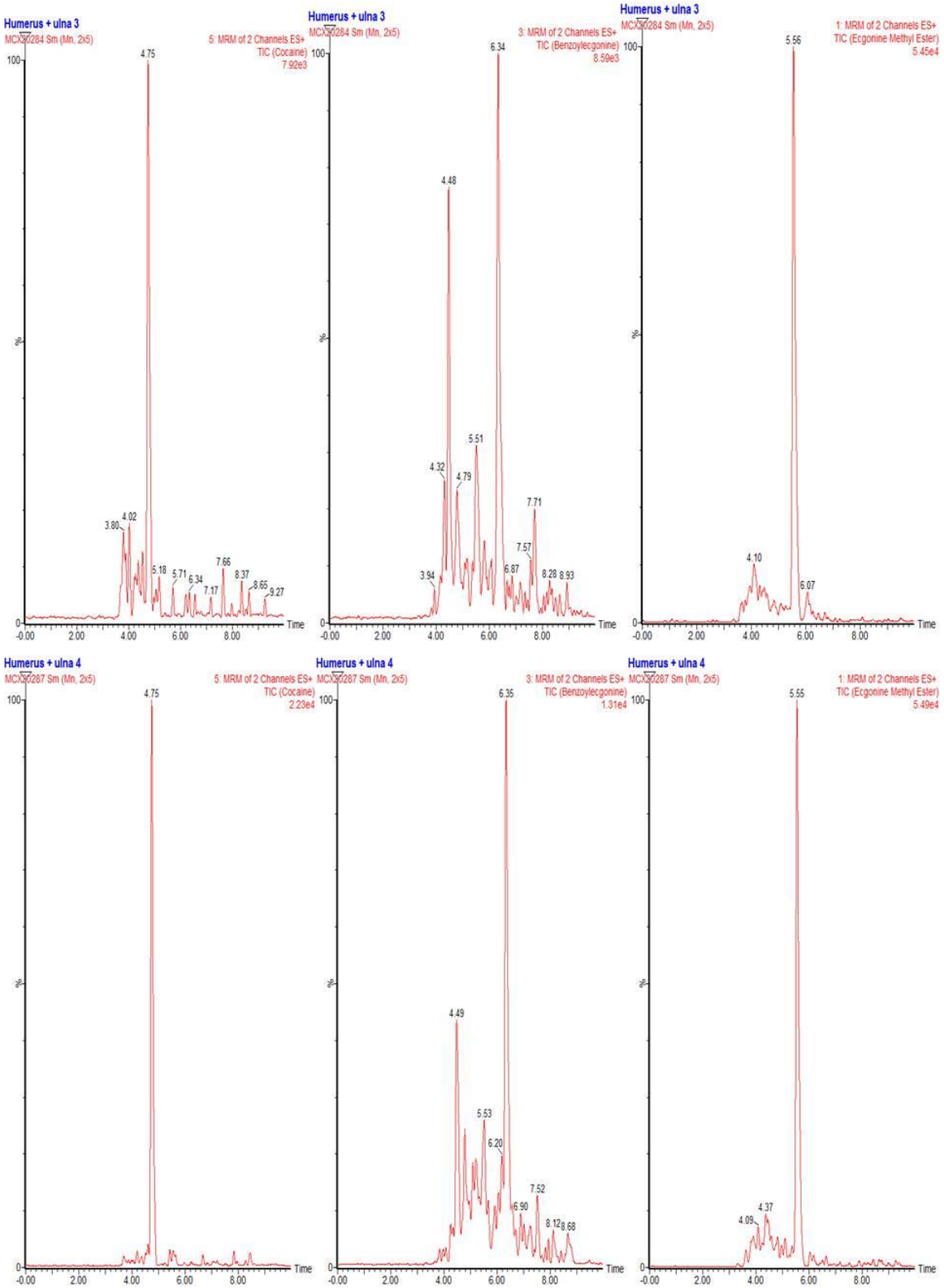
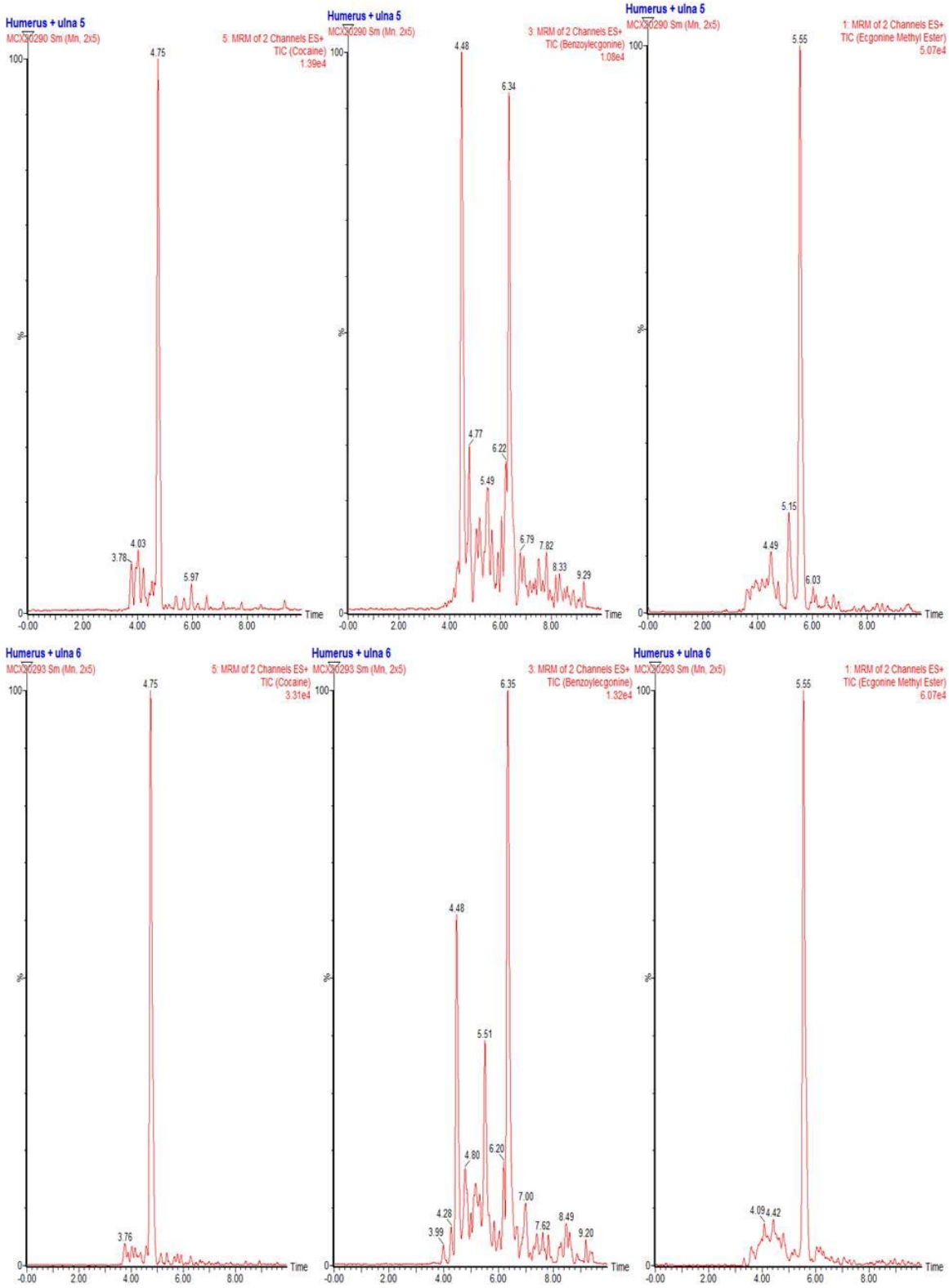
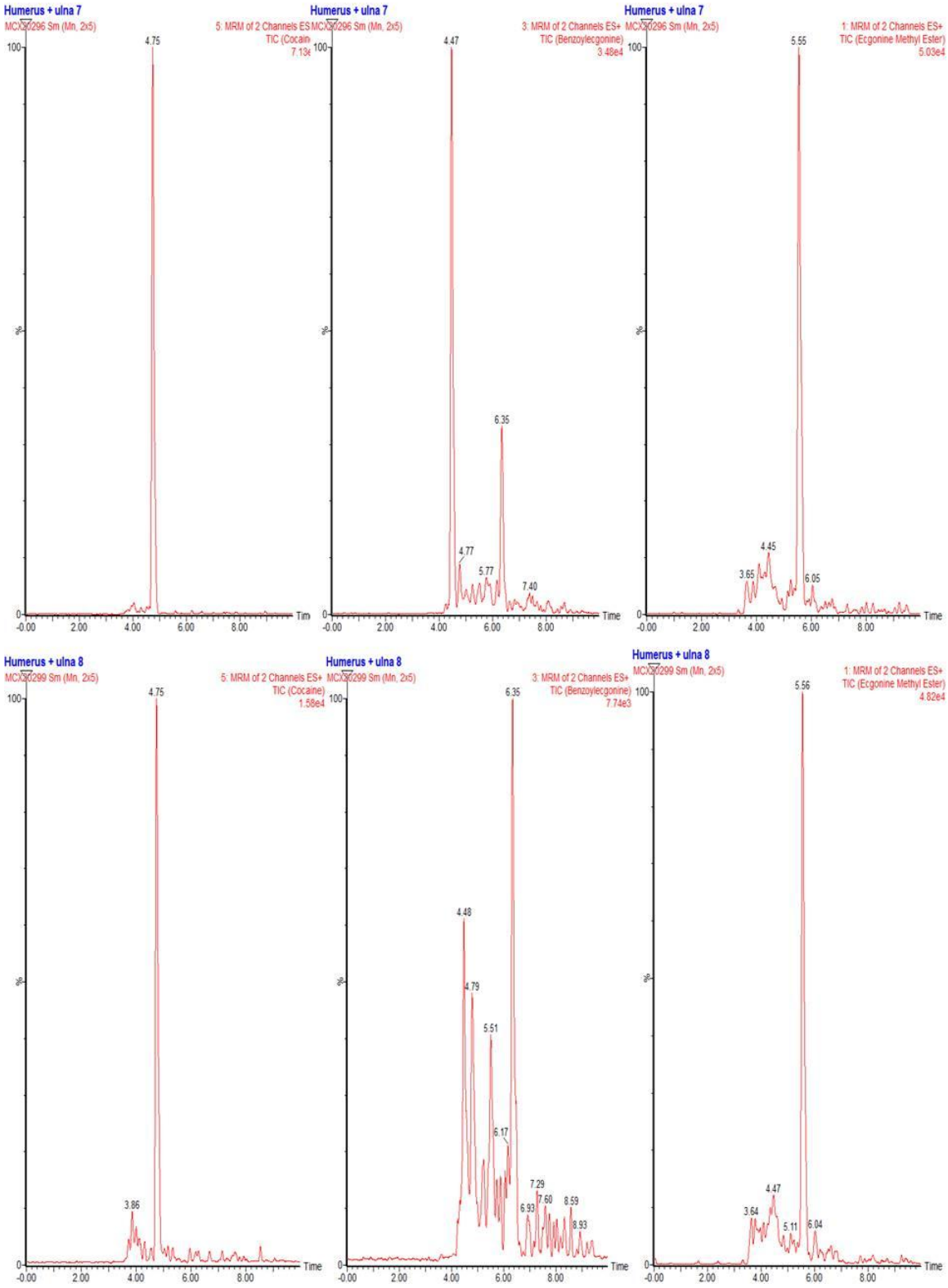
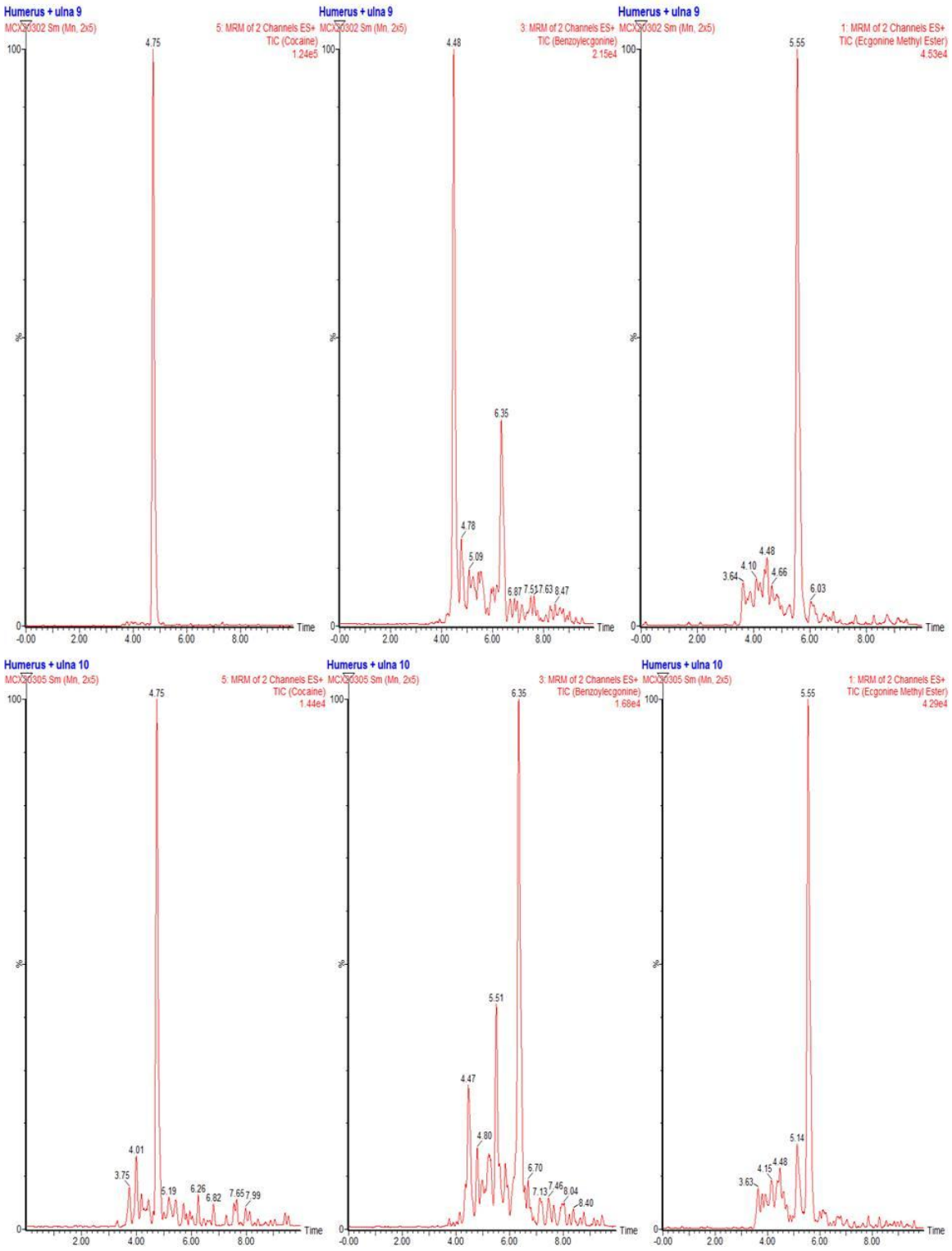
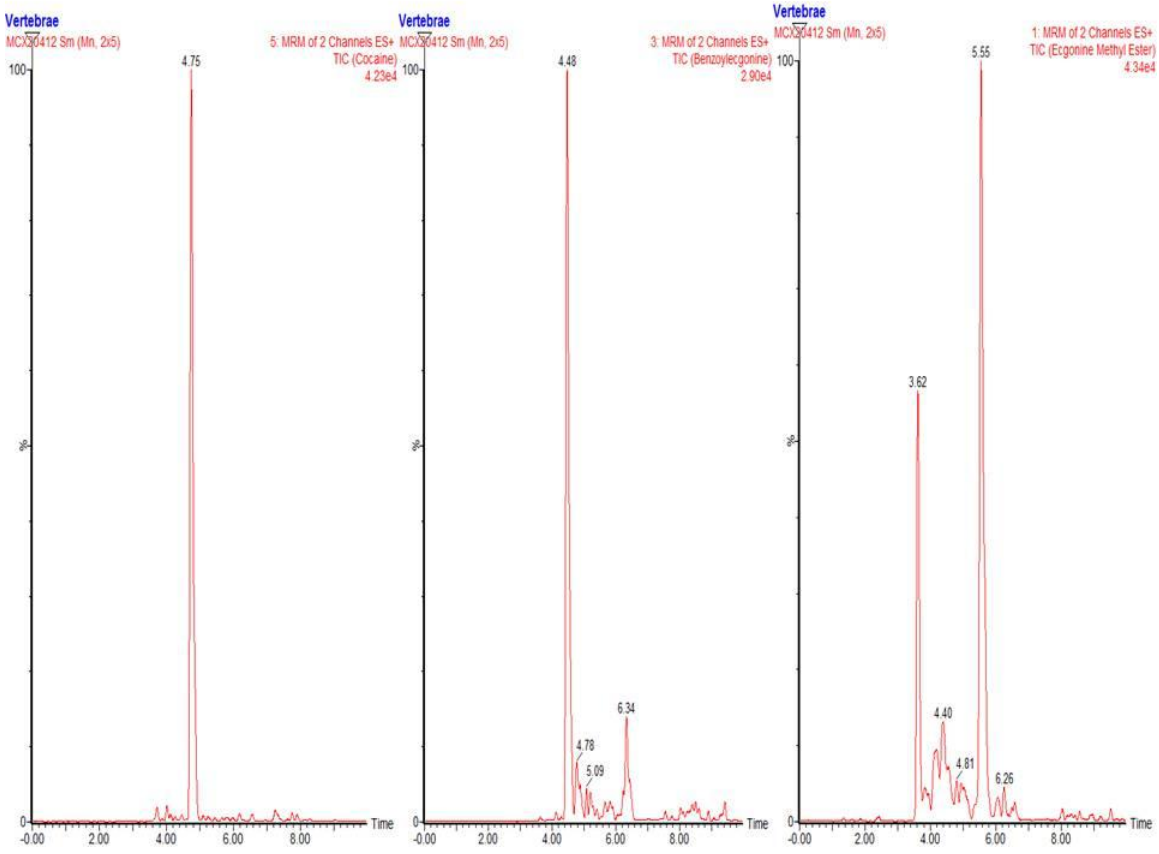

Humerus + ulna 1
MCX20278 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
4.66e4
4.75
Humerus + ulna 1
MCX20278 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.34e4
6.34
5.51
4.48
4.78
6.14
4.25
3.86
6.68
7.98
8.21
8.58
9.07
Humerus + ulna 1
MCX20278 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.15e4
5.55
4.51
4.20
3.61
5.17
Humerus + ulna 2
MCX20261 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.29e4
4.74
3.77
4.37
6.15
Humerus + ulna 2
MCX20261 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
8.08e3
6.35
4.48
4.78
5.49
6.22
5.15
4.35
7.51
7.67
7.15
7.91
9.51
8.29
8.67
Humerus + ulna 2
MCX20261 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.23e4
5.56
4.44
5.10
%
Time
100
0
-0.00
2.00
4.00
6.00
8.00


Humerus + ulna 3
MCX20284 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
7.92e3
4.75
3.80
4.02
5.18
5.71
6.34
7.17
7.66
8.37
8.65
9.27
Humerus + ulna 3
MCX20284 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
8.59e3
6.34
4.48
5.51
4.32
4.79
3.94
6.87
7.71
7.57
8.28
8.93
Humerus + ulna 3
MCX20284 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.45e4
5.56
4.10
6.07
Humerus + ulna 4
MCX20287 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
2.23e4
4.75
Humerus + ulna 4
MCX20287 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.31e4
6.35
4.49
5.53
6.20
6.90
7.52
8.12
8.68
Humerus + ulna 4
MCX20287 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.49e4
5.55
4.09
4.37
Time
%
100
0
-0.00
2.00
4.00
6.00
8.00


Humerus + ulna 5
MCX00290 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.39e4
4.75
4.03
3.78
5.97
Humerus + ulna 5
MCX00290 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.08e4
4.48
6.34
4.77
6.22
5.49
6.79
7.82
8.33
9.29
Humerus + ulna 5
MCX00290 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.07e4
5.55
5.15
4.49
6.03
Humerus + ulna 6
MCX00293 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
3.31e4
4.75
3.76
Humerus + ulna 6
MCX00293 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.32e4
6.35
4.48
5.51
4.80
6.20
7.00
4.28
3.99
7.62
8.49
9.20
Humerus + ulna 6
MCX00293 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
6.07e4
5.55
4.09
4.42
%
Time
0
100
-0.00
2.00
4.00
6.00
8.00


Humerus + ulna 7
MCX50296 Sm (Mn, 2x5)
5: MRM of 2 Channels ES
TIC (Cocain
7.13
4.75
Humerus + ulna 7
MCX50296 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
3.48e4
4.47
6.35
4.77
5.77
7.40
Humerus + ulna 7
MCX50296 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
5.03e4
5.55
4.45
3.65
6.05
Humerus + ulna 8
MCX50299 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.58e4
4.75
3.86
Humerus + ulna 8
MCX50299 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
7.74e3
6.35
4.48
4.79
5.51
6.17
7.29
7.60
8.59
6.93
8.93
Humerus + ulna 8
MCX50299 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
4.82e4
5.56
4.47
3.64
5.11
6.04
Time
%


Humerus + ulna 9
MCX60302 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.24e5
4.75
Humerus + ulna 9
MCX60302 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
2.15e4
4.48
6.35
4.78
5.09
6.87
7.51
7.63
8.47
Humerus + ulna 9
MCX60302 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
4.53e4
5.55
3.64
4.10
4.48
4.66
6.03
Humerus + ulna 10
MCX60305 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
1.44e4
4.75
3.75
4.01
5.19
6.26
6.82
7.65
7.99
Humerus + ulna 10
MCX60305 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
1.68e4
6.35
5.51
4.47
4.80
6.70
7.13
7.46
8.04
8.40
Humerus + ulna 10
MCX60305 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
4.29e4
5.55
5.14
3.63
4.15
4.48
%
Time


Vertebrae
MCX30412 Sm (Mn, 2x5)
5: MRM of 2 Channels ES+
TIC (Cocaine)
4.23e4
4.75
100
%
0
Time
-0.00
2.00
4.00
6.00
8.00
Vertebrae
MCX30412 Sm (Mn, 2x5)
3: MRM of 2 Channels ES+
TIC (Benzoylecgonine)
2.90e4
4.48
4.78
5.09
6.34
Vertebrae
MCX30412 Sm (Mn, 2x5)
1: MRM of 2 Channels ES+
TIC (Ecgonine Methyl Ester)
4.34e4
5.55
3.62
4.40
4.81
6.26

## BIBLIOGRAPHY


1. Skopp G. Preanalytic aspects in postmortem toxicology. Forensic Science International 2004; 142(2-3):75–100.

2. Kegler R, Büttner A, Nowotnik J, Rentsch D. Postmortem investigation of 88-cm-long dreadlocks for drugs of abuse: an unusual case report in the northeast of Germany. Forensic Toxicology 2016; 34(2):419–24.

3. Kronstrand R, Druid H. Hair in Postmortem Toxicology. In: Kintz P, editor. Analytical and Practical Aspects of Drug Testing in Hair. Boca Raton, FL: Taylor & Francis Group, 2007; 223–40.

4. Isenschmid DS. Cocaine. In: Levine B, editor. Principles of Forensic Toxicology. Washington , DC: AACC Press, 2013; 293–316.

5. Fernández MDMR, Fazio VD, Wille SM, Kummer N, Samyn N. A quantitative, selective and fast ultra-high performance liquid chromatography tandem mass spectrometry method for the simultaneous analysis of 33 basic drugs in hair (amphetamines, cocaine, opiates, opioids and metabolites). Journal of Chromatography B 2014; 965:7–18.

6. Watterson JH, Desrosiers NA, Betit CC, Dean D, Wyman JF. Relative Distribution of Drugs in Decomposed Skeletal Tissue. Journal of Analytical Toxicology 2010; 34(8):510–5.

7. Watterson JH, Donohue JP. Relative Distribution of Ketamine and Norketamine in Skeletal Tissues Following Various Periods of Decomposition. Journal of Analytical Toxicology 2011; 35(7):452–8.

8. Watterson JH, Donohue JP, Betit CC. Comparison of Relative Distribution of Ketamine and Norketamine in Decomposed Skeletal Tissues Following Single and Repeated Exposures. Journal of Analytical Toxicology 2012; 36(6):429–33.

9. Watterson JH, Cornthwaite HM. Discrimination Between Patterns of Drug Exposure by Toxicological Analysis of Decomposed Skeletal Tissues. Part II: Amitriptyline and Citalopram. Journal of Analytical Toxicology 2013; 37(8):565–72.

10. Watterson JH, Botman JE. Detection of Acute Diazepam Exposure in Bone and Marrow: Influence of Tissue Type and the Dose-Death Interval on Sensitivity of Detection by ELISA with Liquid Chromatography Tandem Mass Spectrometry Confirmation. Journal of Forensic Sciences 2009; 54(3):708–14.

11. Watterson JH, Desrosiers NA. Examination of the effect of dose-death interval on detection of meperidine exposure in decomposed skeletal tissues using microwave-assisted extraction. Forensic Science International 2011; 207(1-3):40–5.

12. Wiebe TR, Watterson JH. Analysis of tramadol and O-desmethyltramadol in decomposed skeletal tissues following acute and repeated tramadol exposure by gas chromatography mass spectrometry. Forensic Science International 2014; 242:261–5.

13. Cornthwaite HM, Watterson JH. Microwave assisted extraction of ketamine and its metabolites from skeletal tissues. Analytical Methods 2014; 6(4):1142.

14. Cornthwaite HM, Watterson JH. The Influence of Body Position and Microclimate on Ketamine and Metabolite Distribution in Decomposed Skeletal Remains. Journal of Analytical Toxicology 2014; 38(8):548–54.

15. Imfeld AB, Watterson JH. Influence of dose-death interval on colchicine and metabolite distribution in decomposed skeletal tissues. International Journal of Legal Medicine 2015; 130(2):371–9.

16. Desrosiers NA, Watterson JH. The effects of burial on drug detection in skeletal tissues. Drug Testing and Analysis 2010; 2(7):346–56.

17. Desrosiers NA, Watterson JH, Dean D, Wyman JF. Detection of Amitriptyline, Citalopram, and Metabolites in Porcine Bones Following Extended Outdoor Decomposition*. Journal of Forensic Sciences 2011; 57(2):544–9.

18. Delabarde T, Keyser C, Tracqui A, Charabidze D, Ludes B. The potential of forensic analysis on human bones found in riverine environment. Forensic Science International 2013; 228(1-3).

*19.* Substance Abuse and Mental Health Services Administration, *Results from the 2013 National Survey on Drug Use and Health: Summary of National Findings*, NSDUH Series H-48, HHS Publication No. (SMA) 14-4863. Rockville, MD: Substance Abuse and Mental Health Services Administration, 2014.

20. Barroso M, Gallardo E, Queiroz J. Bioanalytical methods for the determination of cocaine and metabolites in human biological samples. Bioanalysis 2009;1(5):977–1000

21. Drummer OH. Postmortem toxicology of drugs of abuse. Forensic Science International 2004; 142(2-3):101–13.

22. Jurado C. Hair Analysis for Cocaine. In: Kintz P, editor. Analytical and Practical Aspects of Drug Testing in Hair. Boca Raton, FL: Taylor & Francis Group, 2007; 95–126.

23. Ettlinger J, Yegles M. Influence of thermal hair straightening on cannabis and cocaine content in hair. Forensic Science International 2016; 265:13–6.

24. Maurer HH. Current role of liquid chromatography–mass spectrometry in clinical and forensic toxicology. Analytical and Bioanalytical Chemistry 2007;388(7):1315–25.

25. Scientific Working Group for Forensic Toxicology. SWGTOX Doc 003: Standard Practices for method validation in forensic toxicology. SWGTOX, 2013.

26. Northrop D. Forensic Applications of High-Performance Liquid Chromatography and Capillary Electrophoresis. In: Saferstein R, editor. Forensic Science Handbook. Upper Saddle River, NJ: Pearson Education, 2002; 41–116.

27. Meyer VR. Practical high-performance liquid chromatography. 4th ed. Chichester: John Wiley, 2004.

28. Neue UD. HPLC columns: theory, technology, and practice. New York: Wiley-VCH, 1997.

29. Bell S. Forensic chemistry. Upper Saddle River, NJ: Pearson Prentice Hall, 2006.

30. Guiochon G, Marchetti N, Mriziq K, Shalliker R. Implementations of two-dimensional liquid chromatography. Journal of Chromatography A 2008; 1189(1-2):109–68.

31. Stoll DR, Li X, Wang X, Carr P, Porter S, Rutan S. Fast, comprehensive two-dimensional liquid chromatography. Journal of Chromatography A 2007; 1168:3–43.

32. Mallet CR, Murphy BP. Multi-Dimensional Chromatography Compendium. Waters Corporation 2015; 1–8.

33. Rainville PD, Simeone JL, Root DS, Mallet CR, Wilson ID, Plumb RS. A method for the direct injection and analysis of small volume human blood spots and plasma extracts containing high concentrations of organic solvents using revered-phase 2D UPLC/MS. The Analyst 2015; 140(6):1921–31.

34. Oertel R, Pietsch J, Arenz N, Zeitz S, Goltz L, Kirch W. Distribution of metoprolol, tramadol, and midazolam in human autopsy material. Journal of Chromatography A 2011;1218(30):4988–94.

35. Mallet C, Botch-Jones S. Illicit Drug Analysis Using Two-Dimension Liquid Chromatography/Tandem Mass Spectrometry. Journal of Analytical Toxicology 2016;40(8):617–27.

**CURRICULUM VITAE**

**Malorie Mella**
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## Education

**Boston University School of Medicine Boston, MA** *Anticipated Jan 2017*
Master of Science, Biomedical Forensic Science, GPA: 3.54

**New York University New York, NY** *Graduated May 2014*
Bachelor of Arts, Anthropology, GPA: 3.427

## Professional Experience

Waters Corporation (Milford, MA) *Aug 2016 – Present*
**Chemist**

- Method development of multidimensional chromatography (2D LC/MS/MS); projects include:
- Conclusion of MS Thesis: Detection of Cocaine and its major metabolites in rodent bone following outdoor decomposition after chronic cocaine administration using 2D-LC/MS/MS
- Analysis of NBOMes in blotter paper and urine using 2D LC/MS/MS
- Analysis of flame retardants in drinking water, river water, and egg using 2D-LC-TOF
- Analysis of pharmaceuticals in human remains of aviation crashes (heart, lung, kidney, brain, liver, spleen, blood, bile)
- Analysis of pharmaceuticals in fish and shrimp using 2D LC/MS/MS
- Perform routine maintenance and troubleshooting on AQUITY 2D-LC/MS/MS instruments

**Research Assistant** *April 2016 – July 2016*

- Method development and validation of multidimensional chromatography (2D LC/MS/MS); projects include:

- Rapid Analysis of Xylazine and Ketamine in Rodent Tissues (heart, lung, kidney, brain, liver, spleen)
- Analysis of drugs of abuse in human remains of aviation crashes (heart, lung, kidney, brain, liver, spleen, blood, bile)

Boston University: Arthritis Center/Rheumatology *Sept 2014-June 2016*

**Research Assistant**

- Primary responsibility is genotyping of laboratory mice
- Genomic DNA extraction (organic/phenol) of rodent tails
- Set up PCR reactions – make primer master mixes, use of thermocycler, run electrophoresis gels to visualize results of PCR—photograph gel under UV illumination

Boston University School of Medicine *Sept 2015-Dec2015*

**Teaching Assistant**

- Work under Dr. Tara Moore – director of BU Forensic Anthropology Program
- Class title: Professional Skills and Thesis Research Development
- Duties: grading papers, student correspondence, office hours

## Research Experience

**Biomedical Forensic Science Master's Thesis** *Sept 2014-Dec 2016*

- Detection of cocaine and its major metabolites (benzoylecgonine, ecgonine methylester) using multidimensional (2D) LC/MS/MS in skeletal remains following outdoor decomposition after chronic cocaine administration
- Developed method to extract and quantify cocaine and metabolites from bone

**Aditu Field School (Navarre, Spain)**

**Archaeology Student –** Aditu Field School (Navarre, Spain) *July 2014*

- Assistance in excavation of medieval human remains in church cemetery located at Zamartze – a monastery near the town of Huarte-Arakil

- Proficiency in identification of fragmented human skeletal remains; as well as distinction between human and non-human skeletal fragments
- Ageing and sexing skeleton techniques (using methods outlined in *Standards* (Buikstra and Ubelaker 1994)

## Leadership

Boston University Forensic Science Society Treasurer
*Appointed October 2014 – Sept 2015*

## Affiliations

Delta-Delta-Nu Forensic Science Honor Society
*September 2016 – Present*